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Sir Charles Tupper Building
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# Studies on Effects of Forest Sprayings with Insecticides, 1952–63, on Fish and Aquatic Invertebrates in New Brunswick Streams: Introduction and Summary[1,2]

By C. J. Kerswill[3]

*Fisheries Research Board of Canada*
*Biological Station, St. Andrews, N.B.*

## ABSTRACT

This introduces a series of papers on fisheries studies associated with operational and experimental forest spraying, mainly with DDT-in-oil insecticide. It outlines: (*a*) the history of the spray program developed to counteract an outbreak of the spruce budworm (*Choristoneura fumiferana*) in New Brunswick; (*b*) administrative arrangements for developing and coordinating a reasonable research and management program concerning the affected fisheries, mainly for Atlantic salmon (*Salmo salar*); (*c*) highlights of field and laboratory studies on effects of insecticide sprayings on caged young salmon and trout (*Salvelinus fontinalis*), on aquatic insect production, on feeding habits of native young salmon, and on population levels of young salmon.

AERIAL FOREST SPRAYING to control an outbreak of the spruce budworm (*Choristoneura fumiferana*) in New Brunswick began in 1952, when 186,000 acres of woodland on the watershed of the Upsalquitch River were sprayed with DDT-in-oil insecticide applied at the rate of 1 lb DDT per 1 U.S. gal per acre (1.12 kg per 4.6 liters per hectare). In 1953 the operational spray area was about 10 times larger, the dosage was reduced to 0.5 lb DDT per 0.5 U.S. gal per acre (0.56 kg per 2.3 liters per hectare), hereafter called ½ lb DDT per acre, and a Crown Company, Forest Protection Limited, was organized to carry out large-scale forest spraying operations in the Province. Operational spraying continued annually through 1958 (Table I) as the budworm hazard spread across New Brunswick in a southeasterly direction. In 1957 the maximum area for any year, nearly 5.7 million acres, was sprayed (Fig. 1).

In 1958 the outbreak area decreased greatly and no aerial spraying operations were considered necessary in 1959. But a sharp resurgence of budworm populations occurred; operational spraying was resumed in 1960, and has continued annually in central New Brunswick. In this same period spruce budworm populations reached outbreak level elsewhere in the Atlantic region, and there were aerial DDT sprayings in the lower St. Lawrence-Gaspé areas of Quebec and in northern Maine.

The interesting history of previous extensive spruce budworm outbreaks in eastern Canada, which are known to have occurred even 150 years ago (Flieger, 1954), and details of all spray operations from 1952 to 1959 are described

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TABLE I. Aerial sprayings of insecticide in New Brunswick, 1952–62, where fisheries personnel made some assessment of effects on aquatic fauna.

| Year | Insecticide (*lb per acre*) | Total acres sprayed (*thousands*) | Acres resprayed |  | Spraying method | Area of fisheries checks |
|---|---|---|---|---|---|---|
|  |  |  | Once | Twice |  |  |
| 1952 | DDT 1 (1 lb/gal/acre) | 186 | 8,000 | – | Stearman planes; irregular blocks | Upsalquitch R. |
| 1953 | DDT ½ (½ lb/½ gal/acre) | 1,810 | 400,000 | – | " | Restigouche and Tobique rivers |
| 1954 | DDT ½ | 1,141 | – | – | " | Miramichi R. |
| 1955 | DDT ½ | 1,124 | – | – | " | " |
| 1956 | DDT ½ | 1,974 | 44,000 | – | " | " |
| 1957 | DDT ½ | 5,691 | – | – | " | " |
| 1958 | DDT ½ | 2,596 | 33,000 | – | Stearman plus a few TBM planes flying "Stearman way"; irregular blocks | " |
|  | DDT 1[a] | 1 | – | – | Stearman; irregular straight-sided blocks | Richibucto R. |
|  | ½ | 3.5 | – | – |  | " |
|  | ¼ | 0.4 | – | – | " | " |
|  | DDD ½[a] | 0.5 | – | – | " | " |
|  | ¼ | 0.5 | – | – | " | " |
| 1959 | DDT ½[a] | 0.5 | – | – | " | Mactaquac R. |
|  | ¼ | 0.32 | – | – | " | " |
|  | Malathion ⅛[a] | 0.39 | – | – | " | " |
| 1960 | DDT ½ | 1,220 | – | – | Stearman and TBM, flying "Stearman way" | Nashwaak and Miramichi rivers |
|  | ¼ | 1,098 | 15,000 | – | " | " |
|  | ⅜ | 307 | – | – | " | " |
| 1961 | DDT ½ | 14 | – | – | TBM; regular blocks, 4⅔ × 4⅔ miles | " |
|  | ¼ | 1,648 | 523,000 | 3,000 | " | " |
|  | DDT ¼[a] | 0.1 | – | – | TBM; strip | Molus R. |
| 1962 | DDT ¼ | 949 | 417,000 | – | TBM; regular blocks, 4⅔ × 4⅔ miles | Miramichi R. |
|  | Phosphamidon 1[a] | 0.1 | – | – | TBM; strip | Hughes Brook, Miramichi R. |
| 1963 | Phosphamidon ½[a] | 22 | 22,000 | – | TBM; strip | McKenzie Brook, Nashwaak R. |

[a] Experimental spraying

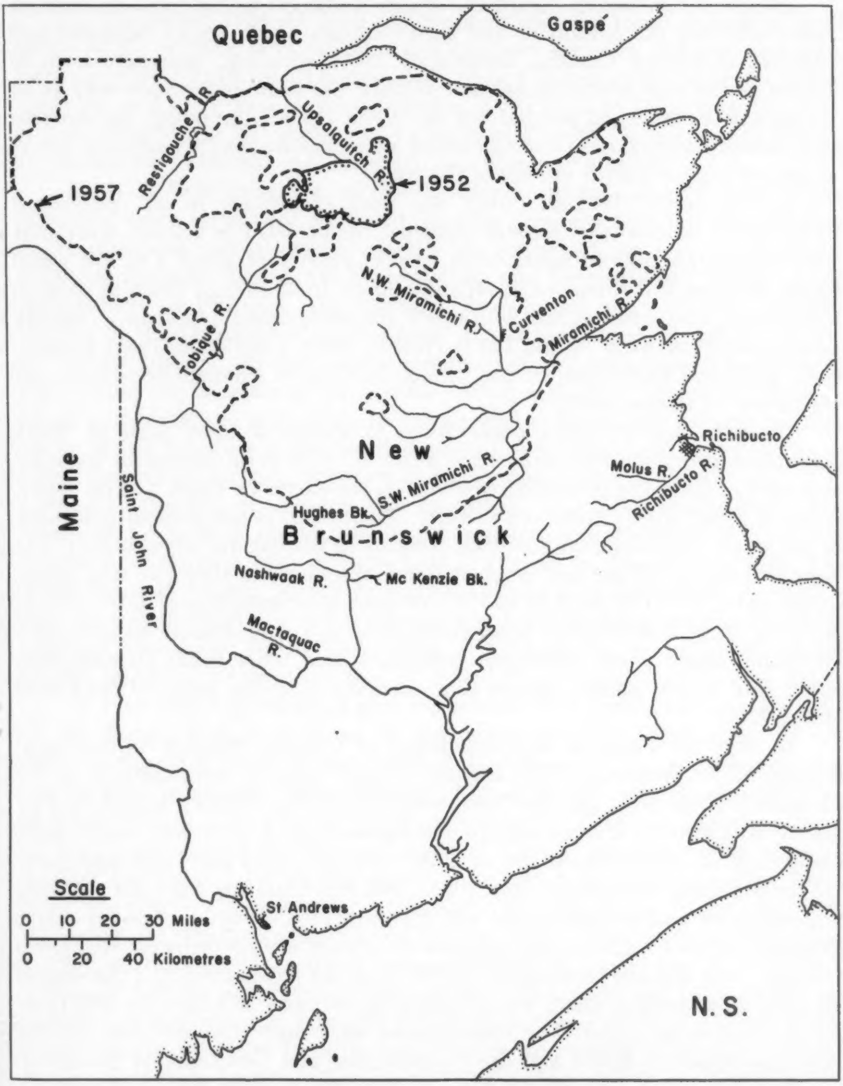
Quebec
Gaspé
1957
1952
Restigouche R.
Upsalquitch R.
N.W. Miramichi R.
Curventon
Miramichi R.
Tobique R.
Maine
Saint John River
New Brunswick
S.W. Miramichi R.
Hughes Bk.
Nashwaak R.
Mc Kenzie Bk.
Mactaquac R.
Molus R.
Richibucto R.
Richibucto
St. Andrews
Scale
0 10 20 30 Miles
0 20 40 Kilometres
N.S.

FIG. 1. Main places and rivers involved in studies of aerial insecticide spraying in New Brunswick, 1952–63. The areas sprayed in 1952 (186,000 acres) and 1957 (5.7 million acres) are outlined to show the extremes in annual spray coverage (several pockets were not sprayed in 1957).

by Webb (1959) and Webb et al. (1961). Macdonald (1964) has summarized later aerial spraying operations in relation to spruce budworm populations. Areas sprayed annually in relation to salmon streams are shown graphically by Elson (1967).

Forest entomologists regard the spruce budworm as the most destructive insect affecting the balsam fir and spruce forests which are so extensive and valuable in eastern Canada. Large-scale DDT spraying, expensive both in dollars and in side effects on fish and wildlife, has been undertaken only as an emergency measure. As pointed out by Webb (1959), the policy has been to recommend spraying only when a further year's defoliation by budworm larvae would seriously threaten the life of the trees.

In the period 1952–63, DDT was used exclusively in all operational sprayings to control budworms in eastern Canada. DDT is a white crystalline solid nearly insoluble in water, with specific gravity of about 1.6. For aerial forest spraying it is dissolved in oil and applied from aircraft flown as close to the ground as is commensurate with safety and navigational problems — usually at an altitude between 50 and 250 ft (Webb, 1959). Hitchcock (1965) summarized published knowledge on the fate of DDT after leaving the aircraft and the effects on stream biota.

Over the past 25 years, observations in waters of many parts of North America during and after aerial application of DDT-in-oil insecticide have led to widely ranging and conflicting opinions on the effects on fishes, and on "safe" levels of application (Rudd and Genelly, 1956). This is not surprising in view of the many variables that condition toxicity in the natural environment. For New Brunswick salmon and trout streams, observations after the first application of DDT at 1 lb per acre in 1952, through later sprayings of DDT at ½ lb per acre, showed that heavy losses could occur in young Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), and other fishes (Keenleyside, 1959), and in the aquatic insects that normally comprise much of their food (Ide, 1957).

In 1950 the Fisheries Research Board of Canada, through its Biological Station at St. Andrews, N.B., started Atlantic salmon investigations in the Miramichi River area, and the Northwest Miramichi tributary was chosen for special attention. On it there were annual assessments of the populations of three size-groups of young salmon (fry or underyearlings, small parr, and large parr) by electroseining on sample areas. Also a two-way counting fence for studying migrations of anadromous fishes was installed at Curventon, 8 miles above tidehead. When woodland over the upper watershed of the Northwest Miramichi tributary was sprayed thoroughly in 1954 from the headwaters to a distance of 40 miles downstream, there was a unique opportunity to assess the effects on salmon and other fishes. The observations were made cooperatively by the Fisheries Research Board and the Conservation and Development Service of the Department of Fisheries of Canada. Besides, a specialist in aquatic invertebrates, Dr F. P. Ide, Department of Zoology, University of Toronto, was called in for consultation. There was no doubt that the 1954 spraying had serious effects on young salmon and their food, as well as on other stream fishes (Kerswill and Elson, 1955). Thenceforth, attention to effects of insecticide spraying was a significant part of the Atlantic salmon investigations of the St. Andrews Station.

In the period 1954–57 both Government and the forest industries were aware of the problem of undesirable side effects of spraying and were seeking a solution. Forest Protection Limited, the Crown Company responsible for actual spraying, and the Forest Biology Division of the Canada Department of Agriculture (now Forest Entomology and Pathology Branch, Department of Forestry) assisted with the fisheries investigations wherever possible.

Early in 1958 formation of the Interdepartmental Committee on Forest Spraying was a significant administrative development that influenced future spray programs and associated studies. It comprised representatives of the Fisheries Research Board, the Conservation and Development Service of the Department of Fisheries, the Forestry Branch and the Canadian Wildlife Service of the Department of Northern Affairs and National Resources, and the Forest Biology Division of the Department of Agriculture. The duties of the Committee as outlined by Prebble (1960) included: (1) review of forest insect outbreaks, the probability of damage to the forest, and hazards to other forms of life resulting from chemical control operations; (2) coordination of recommendations for control action and safeguards to be taken; (3) support of additional research to develop less hazardous insecticide formulations and improved methods of application. The Committee was the result of a federal–provincial conference with industry held in Ottawa on January 10, 1958. The recent losses of Atlantic salmon in eastern Canada were reported at the conference, as was the death of many coho salmon fry and young steelhead trout in northern Vancouver Island, B.C., after DDT spraying to control the black-headed budworm (*Acleris variana*) in June 1957 (Crouter and Vernon, 1959).

In June 1958 experimental spraying studies were carried out cooperatively by the Forest Biology Division of the Department of Agriculture and the Fisheries Research Board, in the vicinity of Richibucto, N.B., to find insecticides less harmful to aquatic fauna than DDT at ½ lb per acre. Besides DDT at three rates (1, ½, and ¼ lb per acre), its analogue DDD was tested on fishes (Table I), and rounel (Korlan) and carbaryl (Sevin), two insecticides reported to show low toxicity for aquatic fauna, were tested on budworms alone. Only DDT was effective in budworm control, and it was effective at all rates provided that adequate coverage was achieved (Fettes, 1960). Further cooperative trials in 1959 in the Mactaquac River area confirmed that reducing DDT concentration to ¼ lb per acre reduced losses of fish. These 1958 and 1959 studies are reported by Kerswill and Edwards (1967). As outlined by Fettes (1960) forest entomologists concluded from the experiments that effective budworm control would result from aerial application of DDT as low as ¼ lb per acre, but the spray had to be evenly distributed to deposit at least 10 drops per square centimetre over the target area.

In 1960 reduction of the DDT dosage to ¼ lb DDT per acre was tested operationally on more than 1 million acres or about half the total spray area of that year, while DDT at ½ lb per acre or ⅜ lb per acre was used on most of the remaining 1.4 million acres (Table I). General results on budworms were good and the fisheries interests hoped that the reduced concentration of DDT at ¼ lb per acre would become standard practice in future operational sprayings. Complications developed in the budworm outbreak, however, involving different kinds of damage to balsam fir and red spruce (Macdonald, 1964), and two applications were sometimes made.

Early forest spraying was commonly done by small single-engine Stearman planes usually used for crop-dusting, and the spray blocks were irregularly shaped and bounded by streams or heights of land. By 1958 the Canadian version of the American Torpedo Bomber, Medium, was declared surplus and became available for forest spraying. A new operational spraying technique was developed using the larger and more powerful planes on regular 4⅔-mile square (55.5 km$^2$) blocks (Flieger, 1964). With the new technique it was not always practicable to shut off spray when the planes flew over rivers traversing the spray block, whereas with the smaller Stearman planes operating on spray blocks usually bounded by streams or land ridges, it was possible to shut off the spray before reaching a stream boundary and reversing direction for the next spray swath. When the new operational technique was used over wide streams in settled areas the resulting mortality of young fish caused public concern and led to useful spraying modifications.

In 1961 and 1962 nearly all operational spraying used DDT at ¼ lb per acre (Table I), but large areas were treated with two sprays at that dosage, spaced about 10 days apart. The first application was to kill budworm larvae on balsam fir early enough to preserve foliage, the second to reduce survivors on red spruce (Macdonald, 1964). Efforts were made to determine the effects on aquatic fauna of this procedure.

There were small-scale experimental sprayings in 1961, 1962, and 1963. The 1961 experiments involved application of DDT at ¼ per lb acre over the Molus River, Richibucto system, mainly for aquatic insect studies. In 1962 the organophosphorus systemic insecticide phosphamidon was applied experimentally on Hughes Brook, Miramichi River, and the survival here of fish and aquatic insects was compared with that on an unsprayed control stream. Studies and surveys comparing the effects of phosphamidon and DDT in reducing budworm populations indicated that they were about equally effective (Macdonald, 1964). Further studies of phosphamidon effects on aquatic insects were made in association with experimental spraying of McKenzie Brook, Nashwaak River, in 1963 (Grant, 1967).

The above outline of spruce budworm control operations and the fisheries–forestry field experiments provides some general background for the succeeding papers of this series, which give comprehensive accounts of several aspects of the studies. Highlights of the results of the different projects as presented in these papers and in accounts already published may be summarized as follows.

1. Forest spraying to control spruce budworms using DDT-in-oil insecticide dispersed by aircraft to deposit ½ lb DDT per acre sometimes caused extensive losses of young wild Atlantic salmon and other stream fishes within several days of extensive spray coverage of the watershed (Elson, 1967; Keenleyside, 1959). Annual population assessments of the three size-classes of young salmon common to New Brunswick streams (underyearlings, small parr, and large parr) with

and without different spray treatments showed the following reductions in abundance after spraying: DDT at ½ lb per acre: underyearlings 90–98%, small parr 70%, large parr 50%; DDT at ¼ lb per acre: underyearlings 50%, small parr 20%, large parr 0%; DDT at ¼ lb per acre applied twice: underyearlings 90–98%, as for a single application of ½ lb per acre (Elson, 1967).

2. Young salmon and trout held in cages in streams in the lower part of sprayed watersheds (Kerswill and Edwards, 1967) consistently suffered high mortality within 3 weeks of spraying with DDT at ½ lb per acre, as compared to unsprayed controls. Spraying with DDT at ¼ lb per acre had less serious effects. Phosphamidon applied at 1 lb per acre had no identifiable effects on fish.

3. Aquatic insects of streams affected by DDT spraying (Ide, 1957, 1967) suffered general cessation of emergence for a 3- to 6-week period after spraying; various insect groups then recovered at different rates. Chironomidae (midges) recovered by late summer of the spray year, but larger forms returned more slowly, for example, some Plecoptera (stoneflies) only after 2 years, Ephemeroptera (mayflies) after 3 years, and Trichoptera (caddisflies) after 4 or more years, depending on location. Some species of mayflies and stoneflies presumably survived spraying while in the egg stage and emerged the summer after spraying. In some cases post-spray total numbers of emerging insects exceeded pre-spray numbers, but the corresponding volume was lower than the pre-spray volume. When phosphamidon was sprayed experimentally instead of DDT (Grant, 1967), no harmful effects on aquatic insects could be detected.

4. Analysis of stomach contents of young salmon (Keenleyside, 1967) showed that after DDT spraying of the Northwest Miramichi watershed their food changed with a change in availability of aquatic insects which normally constitute their diet. For example, before spraying, parr concentrated on Diptera, Trichoptera, and Ephemeroptera, but with reduction of the last two groups they fed on Diptera, snails, worms, and fish.

5. Delayed mortality of young salmon was observed in streams as the water approached freezing in the autumn, after spraying of the watershed in June. In laboratory experiments (Fisheries Research Board of Canada, 1961) specimens from an unsprayed stream and from streams sprayed with DDT at ¼ lb per acre or ½ lb per acre were subjected to gradually lowering temperatures, then held at 1 C. All the unsprayed and ¼-lb DDT specimens survived for a month but over 80% of the ½-lb DDT specimens died within 8 days. Related studies on young salmon by Dr J. M. Anderson and graduate students at the University of New Brunswick, Fredericton, N.B., and Carleton University, Ottawa, Ontario, have elucidated the uptake and detoxification of DDT (Premdas and Anderson, 1963) and the adverse effects of exposure to DDT on temperature responses (Ogilvie and Anderson, 1965).

6. Adult stocks of Atlantic salmon derived from year-classes of young affected by spraying declined in quantity as forecast after results of the extensive 1957 spray operations had been reviewed (Kerswill, 1958). Angling catches are one useful source of data for such analysis; on a stream where all entering fish were counted, the catches were positively correlated with abundance of salmon (Elson and Kerswill, 1964). In sprayed rivers a severe angling slump in 1959–62 was related to effects on young stock of the 1953–58 sprayings of ½ lb DDT per acre. An upsurge in catches in 1963 appeared to be associated with absence of spraying in 1959 combined with other favourable conditions for salmon survival. Operational sprayings were resumed in 1960, but modifications in spraying practice offered promise of less severe damage to adult salmon populations.

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# Fish Losses After Forest Sprayings with Insecticides in New Brunswick, 1952–62, as Shown by Caged Specimens and other Observations[1,2]

BY C. J. KERSWILL[3]

*Fisheries Research Board of Canada*
*Biological Station, St. Andrews, N.B.*

AND

H. E. EDWARDS

*Resource Development Service*
*Department of Fisheries of Canada, Halifax, N.S.*

## ABSTRACT

The survival of young Atlantic salmon and eastern brook trout, held in cages and free-living, was observed in New Brunswick streams inside and outside forested areas sprayed operationally and experimentally from aircraft with DDT and other insecticides for budworm control. DDT-in-oil in a single application at ½ lb/acre caused heavy loss of underyearling salmon and parr within 3 weeks. DDT-in-oil at ¼ lb/acre had no apparent short-term effects on salmon parr, but killed many underyearlings; two applications 10 days apart were about as harmful as a single application at ½ lb/acre. DDD at ½ lb and ¼ lb/acre, and malathion at ⅛ lb/acre, were no more harmful to young salmon than DDT at ¼ lb/acre. Replacing Stearman airplanes with larger aircraft in operational spraying and associated changes in spraying procedure sometimes caused heavy fish losses even for single applications of DDT at ¼ lb/acre. Experimental spraying of phosphamidon-in-water at 1 lb/acre had no apparent harmful effects on young salmon and trout.

Observations at a counting fence across the Northwest Miramichi 12 miles below the lower boundary of the forested area sprayed with DDT at ½ lb/acre in 1954 showed extensive killing of suckers and cyprinids; also, the numbers of these species moving through the fence were abnormally low for several years after spraying.

Wild young salmon were found dead in streams when autumn water temperatures approached freezing after June sprayings of watersheds with DDT.

## INTRODUCTION

AERIAL SPRAYING TO control the spruce budworm (*Choristoneura fumiferana*) as an operational management procedure for spruce and fir forests in New Brunswick began in 1952 (Webb, 1959; Kerswill, 1967). At first the outbreak affected a small area of woodland and only 186,000 acres (1 acre = 0.405 ha), in northwestern New Brunswick were sprayed in 1952. By 1957, however, it had spread over the central part of the Province and spraying was undertaken

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[2] The second in a series of papers on work of the Fisheries Research Board of Canada on effects of forest insecticides on salmon and aquatic insects.

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over 5,691,000 acres, representing 37% of the total forest area of New Brunswick. Later the outbreak subsided, but in the central region budworms still require chemical control. In early years the insecticide was usually DDT-in-oil at 0.5 lb per 0.5 U.S. gal/acre (0.56 kg per 2.3 liters/hectare).

The areas sprayed always included one or more Atlantic salmon (*Salmo salar*) rivers, and from the first the agencies responsible for fisheries were concerned about the effects on production. Results of the 1952 and 1953 sprayings on stream fishes in northwestern New Brunswick were observed by the Department of Fisheries of Canada. Starting in 1954, when the Miramichi River was first affected, the Department of Fisheries and the Fisheries Research Board of Canada together carried out investigations. In addition to regular budworm control operations, from 1958 to 1963 various small-scale experimental sprayings were arranged jointly by forest management and fisheries staffs to test effects of other insecticides and methods of application on both budworms and fishes (Kerswill, 1967).

This paper describes observations on fishes during systematic stream and lake patrols, and on young salmon and trout held in cages and above barriers inside and outside spray areas, as well as miscellaneous observations related to the spraying program which were made at a salmon counting fence on the Northwest Miramichi River and at other places.

The relationship of these investigations to others undertaken in connection with the insecticide spray program is dealt with in the introductory paper of this series (Kerswill, 1967). Places named are located in Fig. 1. Figure 2 shows the locations of cage traps in each of the 6 years they were used.

## METHODS

### PATROLS

In 1952 for periods up to 5 days after spraying, Department of Fisheries staff made observations at 26 check-points scattered over 85% of the watershed of the Upsalquitch River where 186,000 acres were sprayed with DDT at 1 lb/acre (1 lb = 0.454 kg). Six check-points were in shoal areas of small lakes and 20 were on streams including two that had been provided with barrier fences to check drifting dead or moribund fish. About ½ mile (1 mile = 1.609 km) of stream was covered at each point. In 1953 similar patrols were made on the Tobique and Upsalquitch Rivers after spraying of 1.8 million acres of adjacent woodland with DDT at the rate of ½ lb/acre. In early June 1954 at the start of spraying with DDT at ½ lb/acre, Department of Fisheries and Fisheries Research Board staffs joined in regular patrols of the Northwest Miramichi watershed. They also interviewed fishery officers and anglers for reports on fish losses. In later years systematic patrols were discontinued but fishery officers, angling associations, and camp operators complied with requests to report fish losses suspected to be associated with forest spraying.

### FISH HOLDING TESTS

#### CAGED SALMON AND TROUT

In 1954 immediate effects of spraying over the Northwest Miramichi

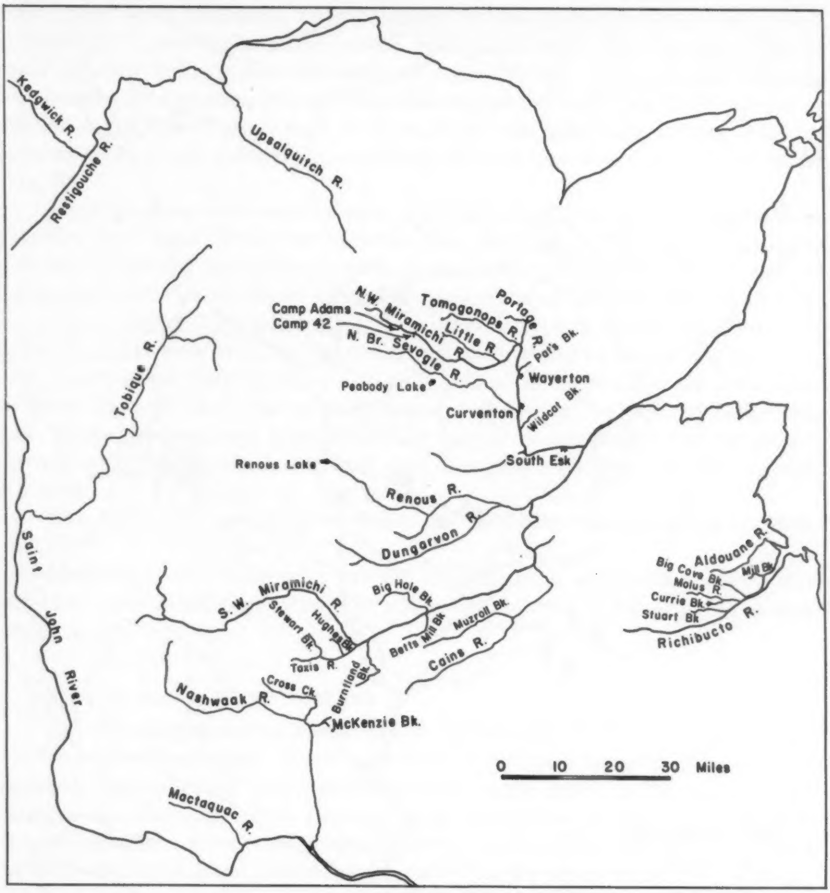
Kedgwick R.
Restigouche R.
Upsalquitch R.
Tobique R.
Camp Adams
Camp 42
N.W. Miramichi
Tomogonops R.
Portage R.
Little R.
N. Br. Sevogle R.
Pat's Bk.
Wayerton
Peabody Lake
Curventon
Wildcat Bk.
South Esk
Renous Lake
Renous R.
Dungarvon R.
Saint John River
S.W. Miramichi R.
Stewart Bk.
Hughes Bk.
Big Hole Bk.
Betts Mill Bk.
Muzroll Bk.
Cains R.
Taxis R.
Burntland Bk.
Cross Ck.
Nashwaak R.
McKenzie Bk.
Mactaquac R.
Aldouane R.
Big Cove Bk.
Mill Bk.
Molus R.
Currie Bk.
Stuart Bk.
Richibucto R.
0 10 20 30 Miles

FIG. 1. Places where observations were made on effects of insecticide sprayings on fishes, New Brunswick.

watershed were assessed by placing similar lots of young salmon in cages inside and outside the spray area several days before the start of spraying. Each cage was 6 × 3 × $2\frac{1}{2}$ ft (1 ft = 30.48 cm) with solid wooden sides, ends of closely spaced $\frac{1}{4}$-inch diam (1 in = 2.54 cm) steel rods, and $\frac{1}{4}$-inch galvanized screening on top and bottom. The cages were positioned on the stream bottom to allow moderate current flow through them and were weighted with a few stones placed inside on the bottom. The stones also provided cover for the fish. The experimental fish were collected in May by seining nearby Miramichi streams. Similar lots of 50 specimens ranging in length from $2\frac{3}{4}$ to $4\frac{1}{2}$ inches were picked at random from the collection (Kerswill and Elson, 1955).

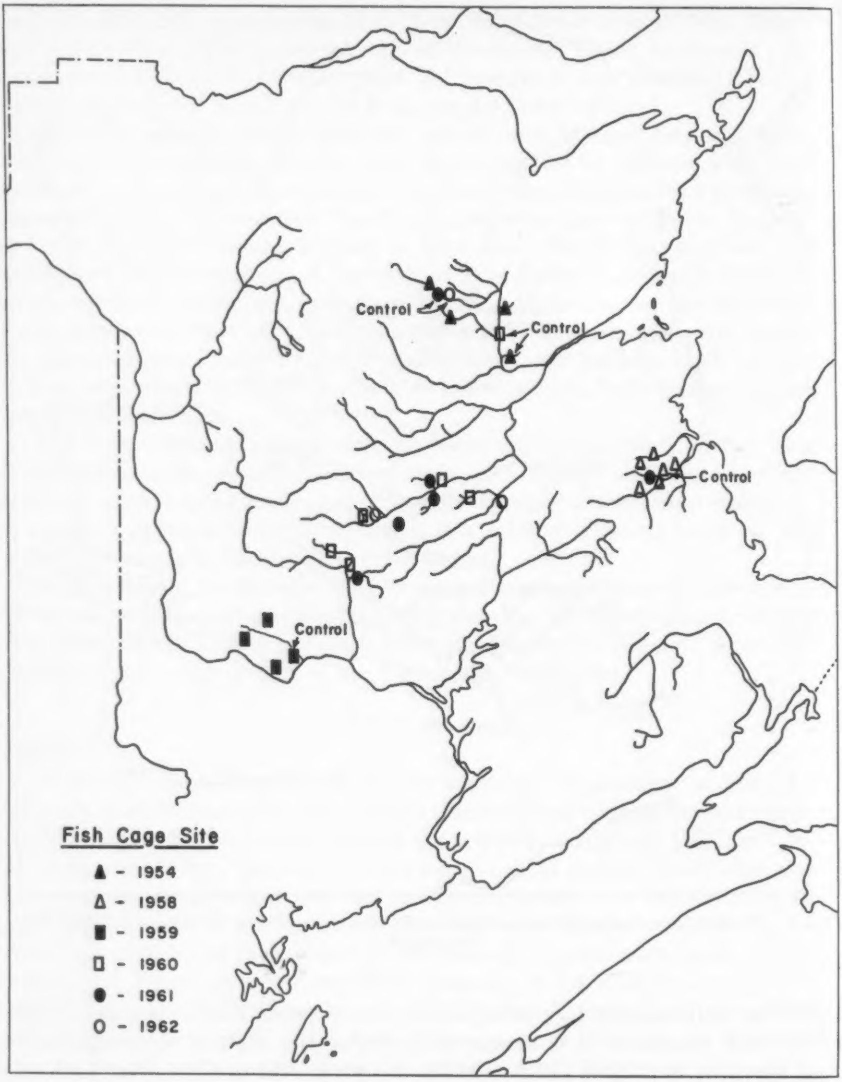
Control
Control
Control
Control
Fish Cage Site
▲ - 1954
△ - 1958
■ - 1959
□ - 1960
● - 1961
○ - 1962

FIG. 2. Locations of fish cages on New Brunswick streams, 1954–62.

In later years a similar method was used in caged-fish tests except for the source of specimens and cage detail. To ensure having sufficient specimens of uniform size, arrangements were made with the Conservation and Development Service (now Resource Development Service), Department of Fisheries, to provide hatchery-produced young salmon and trout. Sometimes, depending on availability of suitable sizes, underyearlings (fry) of salmon and trout were used in addition to yearling or older salmon (parr) and trout. The fry were held in small 1-ft long floating pontoons with solid wooden lids, and sides and bottoms of Fiberglas fly screening placed inside the regular fish cages. In 1960 the size of the fish cages was changed to $4 \times 1\frac{2}{3} \times 1\frac{1}{2}$ ft; the sides and bottom were all covered with $\frac{1}{4}$-inch galvanized screening and the top was a door of plywood (Fig. 3).

The cage sites were selected each year from a plan of spraying operations provided by Forest Protection Limited. Site selection in 1954 was particularly difficult because of inexperience with operational spraying procedures, including general uncertainty about exact date and place of spraying until the day it actually occurred. This difficulty appeared in 1954 well before spraying, and extra cage sites were included to ensure that some would be well inside the spray area, some below but in water draining it, and some outside for unsprayed controls. In later years the procedure was simplified to include only cage sites well inside the proposed spray area but near its lower limit, and unsprayed control sites having similar stream and flow characteristics but well outside the area.

The kinds and quantities of specimens used each year are given in Tables II–VII.

Maximum and minimum water temperatures and water levels were recorded daily at all cage sites. The ranges for sprayed and unsprayed streams were similar.

### FISH ABOVE BARRIER SCREENS

Experimental spraying operations in 1958 on the Richibucto River and in 1959 on the Mactaquac River permitted associated fisheries studies to be planned and executed more precisely than those made in conjunction with operational sprayings. This justified more elaborate pre-spray preparations. Caged-fish tests were supplemented with observations on the survival of hatchery-reared salmon parr released above barrier fences prior to spraying.

Similar lots of 100 specimens were distributed in each of the spray areas and in the unsprayed control streams. The specimens released in both 1958 and 1959 were salmon parr, 2–3 inches long.

To ensure ready access to the planted fish, the streams were cleared of fallen trees, excessive shrubbery, and other debris for about 100 yards (1 yard = 0.914 m) above the barriers. The barriers across a stream consisted of $\frac{1}{2}$-inch vertical steel rods spaced $\frac{3}{8}$ inch apart and supported in wooden frames (Fig. 4). Mesh wire cloth, $\frac{1}{4}$ inch, was laid on top of the rods to prevent specimens from working through. The fences provided accumulation points for dead and dying fish, and prevented movement downstream before spraying started. Dead specimens were collected daily. A daily visual census was made of each cleared section in daylight hours, and occasionally a similar census was made at night using lights.

### Curventon Counting Fence

From 1950 to 1963 the Fisheries Research Board operated a counting fence annually during the open-water season on the Northwest Miramichi River at Curventon, 7 miles above head of tide where the stream was 315 ft wide. It was designed primarily for recording movements of descending smolts and ascending adults of Atlantic salmon. The fence had one up-trap in midstream, where the water depth was greatest, and a down-trap near either bank (Fig. 5). The fish-straining panels consisted of vertical $\frac{1}{4}$-inch round steel rods with $\frac{3}{8}$-inch spaces and few fish smaller than 4 or 5 inches in length were retained. Several other species than salmon were recorded regularly, including the brook trout (*Salvelinus fontinalis*), the white sucker (*Catostomus commersoni*), and the fallfish (*Semotilus corporalis*). Others including shad (*Alosa sapidissima*), alewife (*Alosa pseudoharengus*), smelt (*Osmerus mordax*), blacknose dace (*Rhinichthys atratulus*), and common shiner (*Notropis cornutus*) were recorded occasionally.

Compilation of all available data on fish movements from 1950 to 1958 showed that, other than salmon, only brook trout and white suckers moved past the trap in sufficient quantity and with enough regularity from year to year to show effects of DDT spraying. These data are supplemented by the 1959 counts but later data are omitted, because starting in June 1960 movements of fish were affected by new pollution from base-metal mining operations on the Tomogonops River, which joins the Northwest Miramichi 15 miles above Curventon (Fig. 1).

## RESULTS

### Patrols

#### UPSALQUITCH RIVER, 1952

From June 17 to 30, within 5 days after spraying, observations over an area of about 160,000 acres of watershed showed the following mortalities among fish and other fauna: 189 brook trout of which 12 (1–10 inches long) were in streams and 177 (3–10 inches long) in lakes; 1 salmon parr (3 inches long) in a stream; 244 cyprinids in lakes; 1 sculpin, 3 salamanders, 5 crayfish, 242 mayfly nymphs, and 5 caddisfly larvae, mostly in streams. Of the 12 brook trout found dead in streams, 2 were at barrier fences. Dead organisms in such numbers are rarely if ever observed under natural conditions.

#### TOBIQUE AND UPSALQUITCH WATERSHEDS, 1953

In June, within 5 days after spraying, hundreds of dead salmon parr and 25 dead suckers were found along one 3-mile stretch of the Tobique River. Many dead cyprinids were found in nearby lakes. After freshets in late summer and early fall some dead salmon parr were found in the Kedgwick and Restigouche rivers, an observation not previously recorded in these streams. Precise counts were not made.

### NORTHWEST MIRAMICHI WATERSHED, 1954 (TABLE I)

Many young salmon, trout, and other fishes were found dead after DDT spraying (Table I). Usually more were seen along the stream banks just above watermark than under water. Observations on moribund fish showed that beaching resulted from extreme convulsive activity just before death, combined

TABLE I. Summary of observations by I. M. Jones, Fisheries Research Board (1, below), and N. MacEachern, Department of Fisheries (2, below), during patrols after DDT spraying of Northwest Miramichi River on June 9, 1954; places are shown in Fig. 1.

| Date | | Place | Observations |
|---|---|---|---|
| June | 9 | Hawthorn Pool (Little R. vicinity) | 48 dead parr, some dead frogs (1) |
| | | Peabody Lake | Many dead mayflies on surface; no dead fish (2) |
| | 10 | Call's Pool (Little R. vicinity) | 30 dead parr, others dying (1) |
| | | Mouth of Little R. | 7 dead parr, 2 trout (1 dead, 1 weak) (1) |
| | 11 | Call's Pool | 10 dead parr (1) |
| | | Near Stickney Bridge (Little R. vicinity) | 52 dead parr (1) |
| | 12 | Allison Farm (Portage R. vicinity) | 1 dead parr, 1 dead stickleback (1) |
| | | Stickney Bridge to Curventon | 52 dead parr collected by fisheries officers (1) |
| | | Peabody Lake | Some dead mayflies; no dead fish (2) |
| | 13 | 3-mile stretch below Call's Pool | Angler and guide reported many dead parr, estimated ½ bucket (1) |
| | 14 | Stickney Bridge | 2 dead parr (2) |
| | 16 | Peabody Lake | No dead trout; 1 dead parr, 1 dead frog (2) |
| | 18 | 2-mile stretch above Stickney Bridge | 35 dead yearling parr (avg length, 2½ inches), 1 dead 2-year-old parr (length 4 inches) (2) |
| | 17–18 | Renous Lake | A few dead chub and suckers seen by fisheries officer (1) |
| | 21 | Peabody Lake, Stickney Bridge | No dead fish (2) |
| | 22 | Camp 42 | 3 dead trout (4–6 inches long) (2) |
| | 25 | Peabody Lake | No dead fish (2) |
| | | Stickney Bridge | 8 dead parr, 2 dead cyprinids (2) |
| July | 2 | Camp 42 | 1 salmon fry, 1 parr, 1 trout (length 6 inches) dead (1) |
| | 10 | Stickney Bridge | 12 dead parr (2) |
| | 12 | Camp 42 | 6 dead parr (1) |
| | 14 | Camp 42 | 7 dead parr, 1 dead trout (1) |
| | 21 | Mouth of Little R. | 3 dead parr, 1 dead dace (1) |
| | 27 | Camp 42 | 1 dead trout (1) |
| Aug. | 27 | Little Bald Mountain | 3 dead parr (very thin) (1) |
| Sept. | 2 | Camp 42 | 5 dead parr (very thin) (1) |
| | 4 | Mouth of Little R. | 4 dead parr (1) |
| | 7 | Mouth of Little R. | 7 dead parr (1) |

with repeated swimming to the surface. One 6-inch trout observed on June 10 at the mouth of Little River swam erratically and could be picked up readily. In later years such behaviour of young salmon and trout was a useful criterion of approaching death of caged test specimens.

## Fish Holding Tests

### NORTHWEST MIRAMICHI CAGED FISH, 1954 (TABLE II)

Within 21 days from the start of extensive upstream spraying with DDT at ½ lb/acre, cumulative mortalities of parr ranged from 63 to 91%. Only 2% of the controls had died by that time and the specimens showed no obvious effects of captivity.

Table II. Effects of operational spraying of DDT at ½ lb/acre on caged Atlantic salmon parr (length 2¾–4¼ inches) in Northwest Miramichi River watershed, 1954.

| Cage site | No. of specimens | Start of extensive spraying upstream | Cumulative percentage deaths by intervals after start of extensive spraying | | |
|---|---|---|---|---|---|
| | | | 7 days | 14 days | 21 days |
| Camp 42 | 75 (in 2 cages) | June 17 | 7 | 84 | 91 |
| North Branch Sevogle | 75 (in 2 cages) | June 15 | 1 | 31 | 65 |
| Pat's Brook | 75 (in 2 cages) | June 8 | 1 | 29 | 63 |
| Wildcat Brook (unsprayed control) | 50 (in 1 cage) | – | – | – | 2 |

Because of the spraying procedure there was some uncertainty over the time between spraying and the beginning of fish mortality. As weather permitted, spraying occurred on irregularly shaped blocks about 20–30 miles$^2$ in area (1 mile$^2$ = 2.59 km$^2$), bounded by streams. The earliest possible dates of spraying depended on the stage of development of local budworms, which in turn depended on weather conditions including air temperature and humidity. Sometimes adjacent spray blocks were sprayed 2 or 3 weeks apart. Although it is impossible to state exactly which sprayings caused particular mortalities, sizable groups of specimens died within a few days of the spraying of extensive areas of watershed above the cages. Earlier sprayings over the cage sites but not extending far upstream had no apparent effects on the fish.

### RICHIBUCTO CAGED AND UNCAGED FISH ABOVE BARRIER SCREENS, 1958 (TABLE III)

Observations on effects of three rates of DDT and two of DDD were made in five experimental spray plots in the Richibucto watershed as shown in Fig. 6.

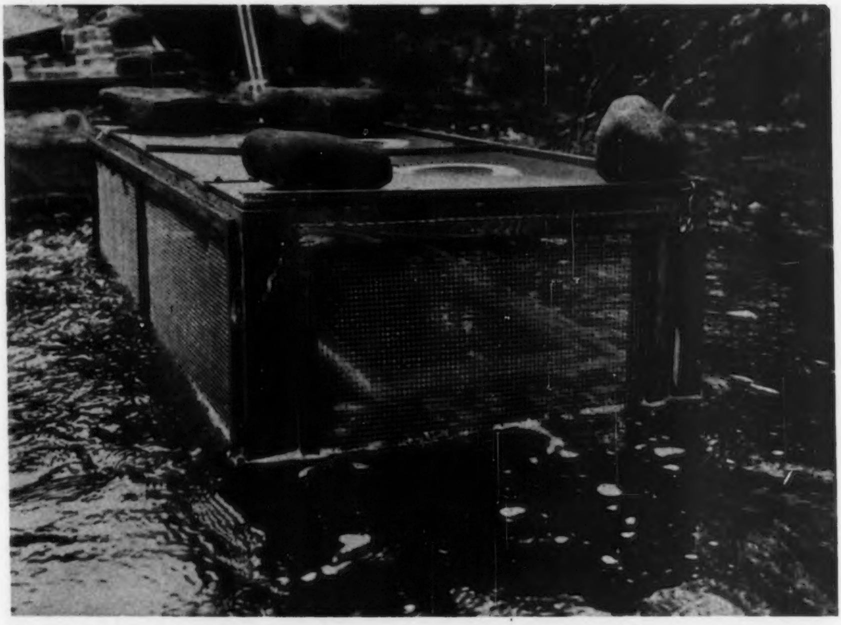

FIG. 3. Cages used to hold young salmon and trout, Big Hole Brook, 1961; note smaller cage for fry floating inside.

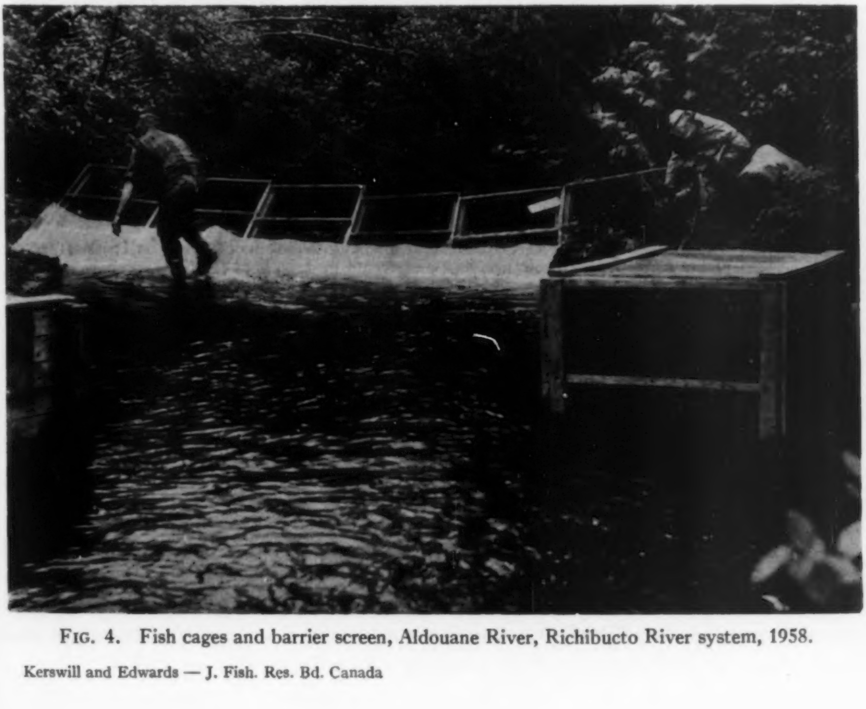

FIG. 4. Fish cages and barrier screen, Aldouane River, Richibucto River system, 1958.

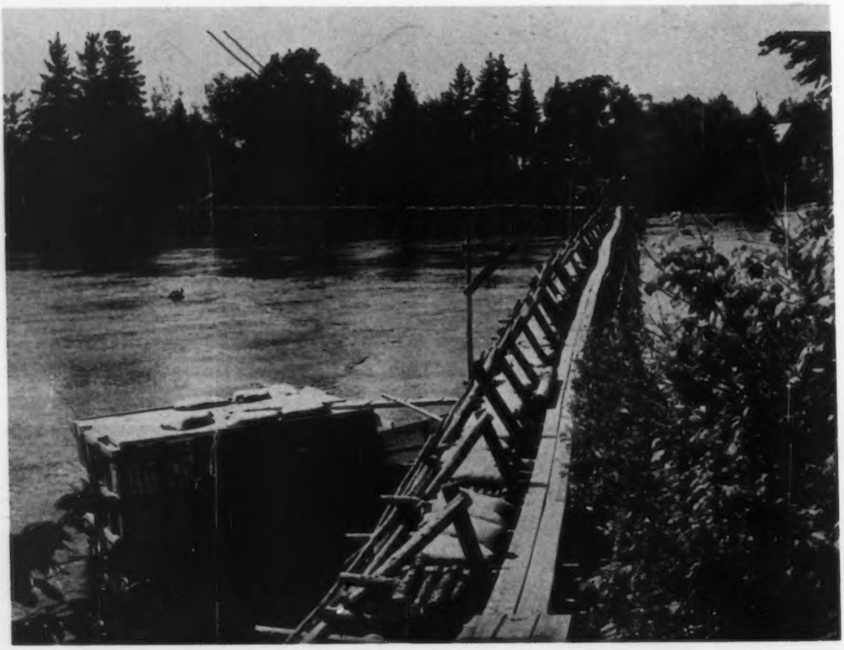

FIG. 5. Two-way counting fence at Curventon, Northwest Miramichi River, 1961; river flows from right to left in picture, and midstream up-trap is at far right.

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TABLE III. Effects of experimental sprayings at different rates of DDT and DDD on hatchery-reared Atlantic salmon yearling parr (length 2–3 inches), caged and free-living, Richibucto River area, 1958.

| Spray application (*lb/acre*) | Cage site | No. of specimens | Date sprayed | Cumulative percentage deaths, to weekends after distribution of parr on June 11 | | | |
|---|---|---|---|---|---|---|---|
| | | | | June 14 | June 21 | June 28 | July 5 |
| Held in cages at lower ends of streams | | | | | | | |
| DDT 1 | Mill Brook | 100 | June 12 | 13 | 26 | 31 | 36 |
| ½ | Aldouane R. | 100 | June 18 | 2 | 17 | 20 | 22 |
| ¼ | Currie Brook | 50 | June 19 | – | – | – | 2 |
| DDD ½ | Stuart Brook | 100 | June 20 | – | – | – | 2 |
| ¼ | Molus R. | 50 | June 21 | – | – | – | 6 |
| Unsprayed control | Big Cove Brook | 50 | – | – | – | – | 2 |
| Released above barrier fences at lower ends of streams | | | | | | | |
| DDT 1 | Mill Brook | 100 | June 12 | 21 | 35 | 36 | 36 |
| ½ | Aldouane R. | 100 | June 18 | – | 10 | 12 | 12 |
| ¼ | Currie Brook | 100 | June 19 | – | – | 2 | 3 |
| ½ | Stuart Brook | 100 | June 20 | – | – | – | – |
| DDD ¼ | Molus R. | 100 | June 21 | – | 1 | 2 | 2 |
| Unsprayed control | Big Cove Brook | 100 | – | – | 1 | 2 | 2 |

In the observation sections, which were under ½ mile long, sprayings of DDT at 1 lb and ½ lb/acre were followed within 2 to 3 weeks by deaths of around 20 to 30% of caged parr and 10 to 35% of free-living parr. In the same streams many other fish were found dead within a few days of spraying. For example, by the end of the third day 34 brook trout, 41 sticklebacks (*Gasterosteus aculeatus*), and 3 sculpins (*Cottus cognatus*) were picked up in the 1-lb DDT/acre stream.

Sprayings of DDT at ¼ lb/acre and DDD at ½ and ¼ lb/acre had no observable effects on caged, introduced, or native fish during a period of 2 or 3 weeks after spraying.

Survival in the control stream was excellent until early August, when observations ended.

### MACTAQUAC CAGED AND UNCAGED FISH ABOVE BARRIER SCREENS, 1959 (TABLE IV)

Observations on caged salmon fry and parr in experimental plots sprayed with DDT at ½ lb and ¼ lb/acre and malathion at ⅛ lb/acre, and in a nearby unsprayed control stream, showed high parr survival. Until July 11, 3 to 4 weeks after spraying, survival of parr was excellent in all cages. For fry, high mortality was apparent within 8 days in the plot sprayed with DDT at ½ lb/acre.

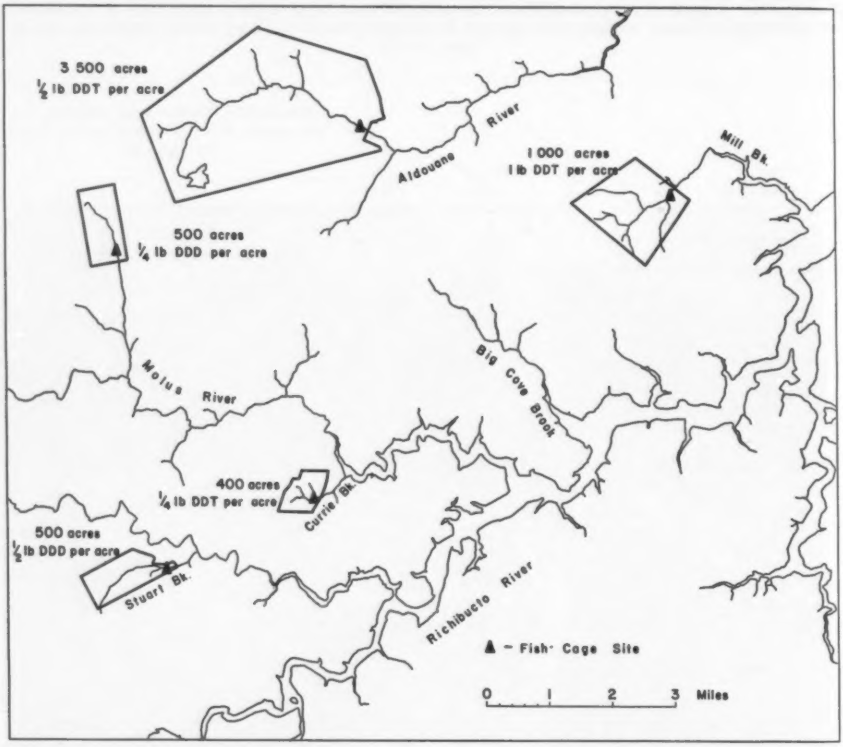
3 500 acres
½ lb DDT per acre
Aldouane River
1 000 acres
1 lb DDT per acre
Mill Bk.
500 acres
¼ lb DDD per acre
Molus River
Big Cove Brook
400 acres
¼ lb DDT per acre
Currie Bk.
500 acres
½ lb DDD per acre
Stuart Bk.
Richibucto River
▲ – Fish Cage Site
0 1 2 3 Miles

FIG. 6. Experimental spray blocks and fish cage sites, Richibucto River system, 1958.

Daytime observations on the lots of 100 free-living planted parr showed no harmful short-term effects of any of the three insecticides. From June 4 to August 10 only three dead specimens were found in the malathion plot (sprayed June 19), none in the $\frac{1}{4}$-lb DDT plot (sprayed June 11), one in the $\frac{1}{2}$-lb DDT plot (sprayed June 19), and one in the unsprayed control stream. Between June 30 and July 11 nighttime observations using gasoline lanterns (Elson, 1942) confirmed that many introduced salmon parr and native trout survived, as follows.

| | $\frac{1}{4}$ lb DDT (Sprayed June 11) | $\frac{1}{2}$ lb DDT (June 19) | $\frac{1}{8}$ lb malathion (June 19) | Unsprayed control |
|---|---|---|---|---|
| Salmon parr | 27 | 59 | 83 | 52[a] |
| Trout | 23 | 29 | 12 | 72 |

[a] View of fish partly hidden by bridge.

TABLE IV. Effects of experimental sprayings at two rates of DDT and one of malathion on caged hatchery-reared fry and parr of Atlantic salmon in streams, Mactaquac River area, 1959.

| Spray application (*lb/acre*) | Cage site | No. of specimens | Date sprayed | Cumulative percentage deaths, to weekends after distribution of fish on June 3 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | June | | | | July | | |
| | | | | 6 | 13 | 20 | 27 | 4 | 11 | 18 |
| Fry (1 inch long) | | | | | | | | | | |
| DDT ½ | Coac Stream | 50 | June 19 | 10 | 10 | 10 | 56 | 62 | 72 | 90 |
| ¼ | Bear Island | 50 | June 11 | – | 8 | 8 | 8 | 8 | 8 | 52 |
| Malathion ⅛ | Hainesville | 50 | June 19 | 6 | 6 | 6 | 6 | 6 | 16 | 54 |
| Unsprayed control | Meadow Brook | 50 | – | 2 | 2 | 2 | 2 | 2 | 6 | 34 |
| Yearling parr (2–3 inches long) | | | | | | | | | | |
| DDT ½ | Coac Stream | 50 | June 19 | – | – | – | 2 | 2 | 2 | 10 |
| ¼ | Bear Island | 54 | June 11 | – | – | – | – | – | – | – |
| Malathion ⅛ | Hainesville | 50 | June 19 | – | – | – | – | – | – | – |
| Unsprayed control | Meadow Brook | 50 | – | – | – | – | – | – | – | 4 |

### NASHWAAK AND MIRAMICHI CAGED FISH, 1960 (TABLE V)

The 1960 operational spraying program provided an opportunity to compare effects on salmon parr and fry and trout fingerlings of DDT at ½ lb/acre, ⅜ lb/acre, and ¼ lb/acre. For this one year the day-by-day deaths of salmon parr and fry and the spray dates are shown graphically (Fig. 7).

By June 18, 8 days or longer after the first spraying, there were considerable differences in survival among the various lots of salmon parr and trout fingerlings, losses being lowest in the controls. The mortalities by June 18 were roughly proportional to the amount of DDT applied per acre. Differences in survival of salmon fry were not as marked until a week later.

Stewart and Muzroll brooks (Miramichi system) were scheduled for the same spray treatment, ¼ lb DDT/acre, but by June 18 mortalities at Stewart Brook were much heavier than at Muzroll Brook and similar to those at upper Cross Creek (Nashwaak system) sprayed at ½ lb DDT/acre. Later it was learned that Stewart Brook had received a heavier dosage than was planned, of the order of ½ lb DDT/acre.

### NASHWAAK AND MIRAMICHI CAGED FISH, 1961 (TABLE VI)

Within about 3 weeks of the start of the single application of ¼ lb DDT/acre at McKenzie (Nashwaak system) and Burntland (Miramichi system) brooks, salmon parr suffered about 40% mortality, as at Muzroll Brook in 1960 after

TABLE V. Effects of operational sprayings at different rates of DDT on caged hatchery-reared salmon and trout, Nashwaak and Miramichi streams, 1960.

| | | | | Cumulative percentage deaths, to weekends after distribution of fish on May 25–26 | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | June | | | |
| Spray application (*lb/acre*) | Cage site | No. of specimens | Dates sprayed | May 28 | 4 | 11 | 18 | 25 |
| Yearling parr (3–3½ inches long) | | | | | | | | |
| ½ | Cross Creek (upper) | 100 | May 28, June 8, 9, 10 | 3 | 14 | 44 | 82 | 88 |
| ½ | Cross Creek (lower) | 100 | May 28, June 8, 9 | – | 10 | 15 | 24 | 30 |
| ⅜ | Big Hole Brook | 100 | June 10, 11, 12 | – | 1 | 9 | 54 | 61 |
| ¼ (actually ca. ½) | Stewart Brook | 100 | June 9, 10 | – | 1 | 5 | 74 | 88 |
| ¼ | Muzroll Brook | 100 | May 30, June 7, 8, 9 | – | 11 | 18 | 38 | 47 |
| Unsprayed control | Northwest Miramichi (Curventon) | 100 | – | – | 5 | 12 | 17 | 22 |
| Fry (1½ inches long) | | | | | | | | |
| ½ | Cross Creek (upper) | 100 | May 28, June 8, 9, 10 | – | 5 | 5 | 19 | 85 |
| ½ | Cross Creek (lower) | 100 | May 28, June 8, 9 | 1 | 4 | 12 | 28 | 61 |
| ⅜ | Big Hole Brook | 100 | June 10, 11, 12 | 6 | 11 | 14 | 15 | 22 |
| ¼ (actually ca. ½) | Stewart Brook | 100 | June 9, 10 | 2 | 2 | 4 | 53 | 96 |
| ¼ | Muzroll Brook | 100 | May 30, June 7, 8, 9 | – | 7 | 7 | 20 | 26 |
| Unsprayed control | Northwest Miramichi (Curventon) | 100 | – | – | – | – | 2 | 5 |
| Trout fingerlings (3–5 inches long) | | | | | | | | |
| ½ | Cross Creek (upper) | 50 | May 28, June 8, 9, 10 | – | – | 22 | 42 | 57 |
| ½ | Cross Creek (lower) | 50 | May 28, June 8, 9 | – | – | 2 | 14 | 14 |
| ⅜ | Big Hole Brook | 50 | June 10, 11, 12 | – | – | 2 | 14 | 14 |
| ¼ (actually ca. ½) | Stewart Brook | 50 | June 9, 10 | 2 | 2 | 2 | 26 | 40 |
| ¼ | Muzroll Brook | 50 | May 30, June 7, 8, 9 | – | 6 | 10 | 11 | 22 |
| Unsprayed control | Northwest Miramichi (Curventon) | 50 | – | – | – | – | – | – |

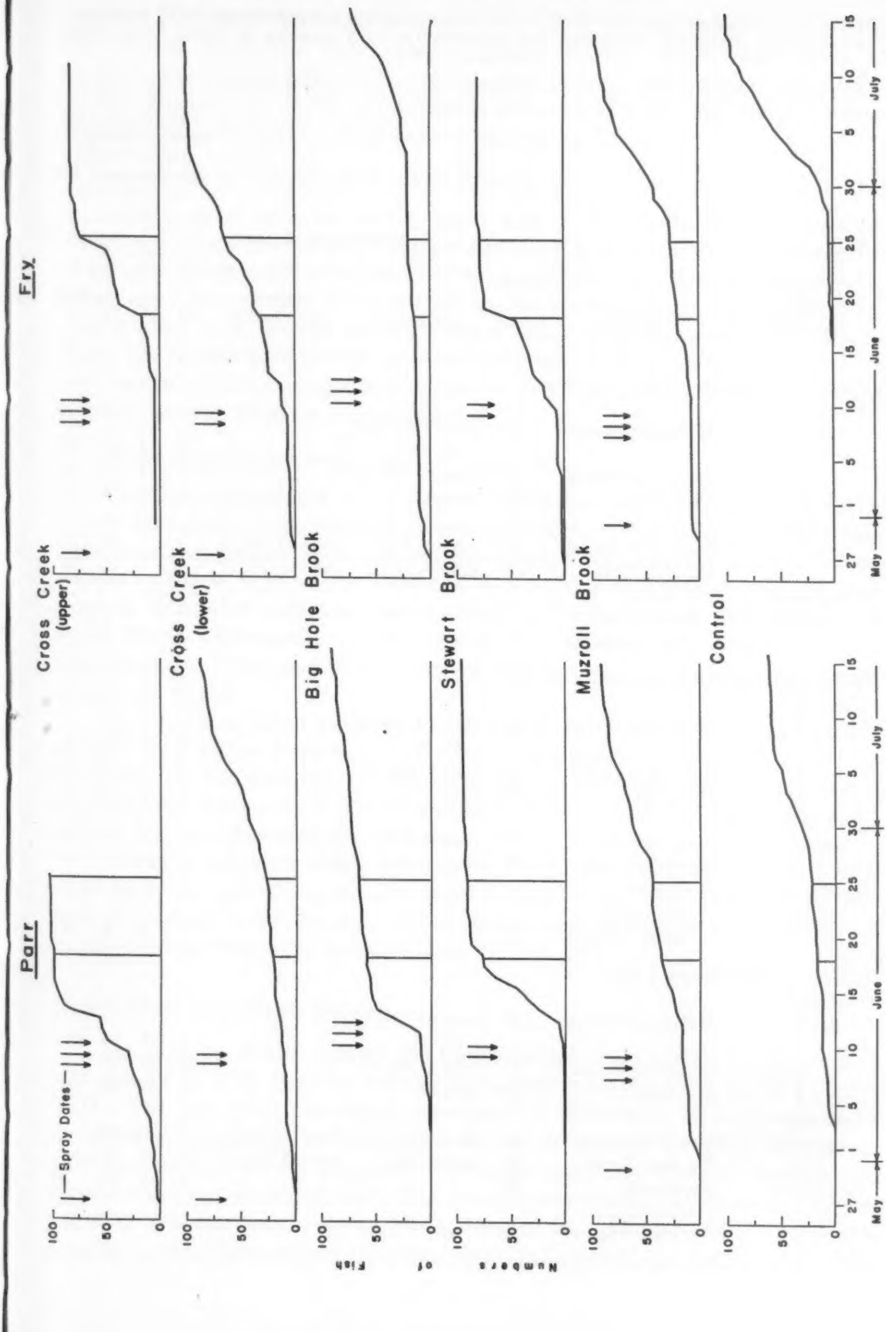

FIG. 7. Cumulative mortalities of salmon parr and fry held in cages, Nashwaak and Miramichi Rivers, 1960.

TABLE VI. Effects on caged hatchery-reared salmon and trout of operational DDT spraying on Nashwaak and Miramichi watersheds and experimental DDT spraying of Molus River (Richibucto area), 1961.

| | | | | | Cumulative percentage deaths, to weekends after distribution of fish on June 4–6 | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Dates sprayed | | June | | | |
| Spray application (*lb/acre*) | Cage site | No. of specimens | First application | Second application | 10 | 17 | 24 | July 1 |
| Salmon parr (average length 3½ inches) | | | | | | | | |
| One application ¼ | McKenzie Brook | 100 | June 11, 12, 15, 16, 19 | | 5 | 24 | 37 | 52 |
| ¼ | Burntland Brook | 90 | June 17, 18, 19 | | – | 1 | 38 | 46 |
| ¼ | Molus R. | 94 | June 11 | | – | 4 | 9 | 25 |
| Two applications ¼ | Betts Mill Brook | 81 | June 5, 8, 9 | June 20, 21, 23 | – | 7 | 90 | 96 |
| ¼ | Big Hole Brook | 100 | June 5, 7, 8 | June 19, 20, 21 | 6 | 9 | 85 | 100 |
| Unsprayed control | Northwest Miramichi (Camp Adams) | 101 | – | | 4 | 8 | 9 | 10 |
| Trout fingerlings (average length 5¼ inches) | | | | | | | | |
| One application ¼ | McKenzie Brook | 50 | June 11, 12, 15, 16, 19 | | – | 6 | 6 | 6 |
| ¼ | Burntland Brook | 45 | June 17, 18, 19 | | – | – | – | – |
| ¼ | Molus R. | 48 | June 11 | | – | – | – | 6 |
| Two applications ¼ | Betts Mill Brook | 54 | June 5, 8, 9 | June 20, 21, 23 | – | – | – | 7 |
| ¼ | Big Hole Brook | 50 | June 5, 7, 8 | June 19, 20, 21 | – | – | – | – |
| Unsprayed control | Northwest Miramichi (Camp Adams) | 50 | – | | 4 | 4 | 4 | 4 |
| Trout fry (average length 1 inch) | | | | | | | | |
| One application ¼ | McKenzie Brook | 50 | June 11, 12, 15, 16, 19 | | – | 78 | 96 | 100 |
| ¼ | Burntland Brook | 33 | June 17, 18, 19 | | 28 | 28 | 100 | – |
| ¼ | Molus R. | 23 | June 11 | | – | 39 | 39 | 86 |
| Two applications ¼ | Betts Mill Brook | 47 | June 5, 8, 9 | June 20, 21, 23 | 9 | 30 | 100 | – |
| ¼ | Big Hole Brook | 50 | June 5, 7, 8 | June 19, 20, 21 | – | 9 | 99 | 99 |
| Unsprayed control | Northwest Miramichi (Camp Adams) | 50 | – | | 4 | 10 | 20 | 20 |

the same treatment. Two applications of ¼ lb DDT/acre at Betts Mill and Big Hole brooks (Miramichi system) were followed by much higher parr losses, of the same order as for ½ lb DDT/acre at upper Cross Creek and Stewart Brook in 1960. The relatively large trout fingerlings survived well at all sites but many trout fry died after even a single spray application.

### MOLUS RIVER CAGED FISH, 1961 (TABLE VI)

Experimental spraying of the lower end of the Molus River watershed (Richibucto system) with DDT applied once at ¼ lb/acre was arranged specially for aquatic insect studies. It provided an opportunity to compare the survival of fish specimens similar to those held in the Nashwaak and Miramichi operational spray area. Except for much lower losses of salmon parr in the Molus River, the results were similar to those obtained in the operational spray area and confirmed that a single application of DDT at ¼ lb/acre is less harmful to young salmon than two applications.

### MIRAMICHI CAGED FISH, 1962 (TABLE VII)

The 1962 operational spray plan included complete coverage with DDT at ¼ lb/acre of the Cains River watershed; it was the only part of the Miramichi watershed that had not been sprayed previously. The survival of caged young salmon and trout held at the lower end of the Cains River was compared with survival of similar specimens held in Hughes Brook, a small tributary of the Taxis River (Miramichi system) which was sprayed experimentally with phosphamidon. The unsprayed control was at Camp Adams on the Northwest Miramichi River.

On the Cains River all trout fry died within 2 weeks and all salmon parr and fry died within 3 weeks of the start of spraying on June 4 with DDT at ¼ lb/acre. By the time that all fish were dead in the Cains River cages, losses at the Camp Adams control had reached only 3, 17, and 4% for salmon parr, salmon fry, and trout fry respectively.

Losses of salmon fry and trout fry at Hughes Brook in the 2-week period after spraying with phosphamidon were negligible. The loss of 35% of the salmon parr sample is attributable to inferior condition of this one lot of specimens, as noted when they were received from the hatchery.

## Curventon Counting Fence

For both trout and suckers the total number of up and down movements was greater in 1954, the first year of Miramichi spraying, than in 1953 (Table VIII). The very high downward movement of suckers in 1954 was probably an effect of spraying. The trout data do not show such an effect; this agrees with the observation by Langford (1949) that speckled trout of yearling size have a lower sensitivity to DDT than the common sucker. Repetition of spraying over the Northwest Miramichi watershed in 1956 and 1957, though less extensive, may have helped to reduce up-movements of the fish through 1958.

TABLE VII. Effects of operational DDT spraying and small-scale experimental phosphamidon spraying on caged hatchery-reared salmon and trout, 1962.

| | | | | Cumulative percentage deaths, to weekends after distribution of fish (June 2 on Cains R., June 6–7 on Hughes Brook and control) June | | |
|---|---|---|---|---|---|---|
| Spray application (*lb/acre*) | Cage site | No. of specimens | Dates sprayed | 9 | 16 | 23 |
| Parr (average length 2¼ inches) | | | | | | |
| DDT ¼ | Cains R. | 50 | June 4–16 (11 days' spraying) | 2 | 74 | 100 |
| Phosphamidon 1 | Hughes Brook | 100 | June 9 | 1 | 14 | 35 |
| Unsprayed control | Northwest Miramichi (Camp Adams) | 100 | – | 1 | 2 | 3 |
| Fry (average length 1¼ inches) | | | | | | |
| DDT ¼ | Cains R. | 50 | June 4–16 | – | 88 | 100 |
| Phosphamidon 1 | Hughes Brook | 100 | June 9 | – | 3 | 7 |
| Unsprayed control | Northwest Miramichi (Camp Adams) | 100 | – | 1 | 6 | 17 |
| Trout fry (average length 1 inch) | | | | | | |
| DDT ¼ | Cains R. | 50 | June 4–16 | 48 | 100 | 100 |
| Phosphamidon 1 | Hughes Brook | 50 | June 9 | – | – | 6 |
| Unsprayed control | Northwest Miramichi (Camp Adams) | 50 | – | 2 | 4 | 38 |

In 1955, the year after the first spraying, both species showed very few upstream movements and this continued through 1958, followed by a marked increase of both species in 1959. Down movements of both trout and suckers were fewer in 1955 than in 1954 but there was no obvious pattern related to spray history for the later years. Too little is known about the life history and behaviour of either species in Miramichi waters to interpret the data reliably, but it is assumed that the extent of their movements through the counting traps is an indication of their abundance. This is particularly true of brook trout, many of which are anadromous or "sea run" and these account for most of the up-trap counts of trout.

Shortly after the start of the 1954 spraying over the Northwest Miramichi, dead fish began to accumulate at the Curventon fence (Table IX). Although the immediate effects of spraying on suckers and cyprinids were seen within 2 days at a distance of 12 miles by river below the spray boundary, such was not the case for brook trout. It is possible, however, that trout were affected

TABLE VIII. Movements of brook trout and white suckers through Curventon counting fence, Northwest Miramichi River, totalled by years, 1953–59.

| | DDT spraying of watershed | Brook trout | | White suckers | |
|---|---|---|---|---|---|
| | | Upward | Downward | Upward | Downward |
| 1953 | None | 254 | 237 | 608 | 290 |
| 1954 | Heavy upriver | 406 | 254 | 1010 | 2982 |
| 1955 | None | 7 | 75 | 30 | 160 |
| 1956 | Some both up- and downriver | 24 | 115 | 30 | 127 |
| 1957 | Some | 2 | 646 | 10 | 470 |
| 1958 | None | 46 | 185 | 20 | 280 |
| 1959 | None | 384 | 317 | 124 | 325 |

but either succumbed sooner or possibly were deposited in unsearched places nearer the spray area through a difference in behaviour upon being subjected to insecticide. Keenleyside (1959) reports decreases in resident trout, cyprinids, and suckers in the Northwest Miramichi after spraying.

## MISCELLANEOUS OBSERVATIONS OF FISH LOSSES

Many reports of fish deaths in New Brunswick waters during years of DDT spraying have been brought to our attention. Usually they originated from observations by fishery officers and anglers, and the reports contained enough detail to show that young salmon losses had indeed occurred within a few days or weeks of spraying. In most cases the extent of losses could not be assessed from the reports. But sometimes casual observations that seemed insignificant at the time assumed more meaning as investigations progressed.

In 1962 considerable public attention was attracted by losses of young fish in the lower settled stretches of the Miramichi River, related to a change in spraying technique. Early forest spraying in New Brunswick featured use of small Stearman aircraft flying in pairs and covering irregularly shaped spray blocks bounded by rivers and heights of land. By 1959 war surplus TBM (Torpedo Bomber, Medium) planes, larger and faster than Stearmans, became available and in 1961 a new spraying technique was developed for them (Flieger, 1964). It involved spraying by two planes over regularly shaped spray blocks and it became impracticable to shut off the spray when the planes flew over open stretches of water, as had been done using the Stearman aircraft.

## LATE-SEASON MORTALITIES

There have been several reports of late-season deaths of young salmon in areas sprayed with DDT in June of the same year. For example, a Department of Fisheries technician (H. W. Coates, personal communication) reported that

TABLE IX. Numbers of live and dead fish other than salmon at Curventon counting fence before and after start on June 9, 1954, of DDT spraying over woodland more than 12 miles above fence site.

| | In two down-traps combined | | | | | | |
|---|---|---|---|---|---|---|---|
| | Brook trout | | White suckers | | Cyprinids | | |
| Date(s) | Alive | Dead | Alive | Dead | Alive | Dead | Dead on fence racks |
| May 25–31 | 91 | 0 | 281 | 0 | 200 | 0 | 0 |
| June 1–7 | 65 | 0 | 219 | 0 | 308 | 0 | 0 |
| 8–10 | 18 | 0 | 92 | 0 | 415 | 0 | 0 |
| 11 | 0 | 0 | 64 | 25 | 135 | Many | 31 suckers and cyprinids |
| 12–14 | 0 | 0 | 146 | 1 | 580 | 0 | 23 suckers, 6 cyprinids |
| 15–21 | 5 | 0 | 209 | 6 | 553 | 3 | 3 cyprinids |
| 22–28 | 1 | 0 | 96 | 8 | 478 | 193 | 0 |
| June 29–July 5 | 0 | 0 | 268 | 31 | 258 | 32 | 0 |
| July 6–31 | 2 | 0 | 787 | 238 | 132 | 11 | 0 |
| Total | 182 | 0 | 2162 | 309 | 3059 | 239+ | 63 |

on October 27, 1954, two protection officers found a great many dead salmon parr along the upper waters of the Northwest Miramichi and Sevogle rivers. At one place, near the main road bridge over the North Branch Sevogle River, they found 50 dead parr. It was at the onset of cold weather when the water temperature approached freezing for the first time.

A similar observation of autumn mortalities after June DDT spraying was made by C. J. Kerswill on October 30, 1957, during a search of the Northwest Miramichi and lower Sevogle rivers in the vicinity of the Curventon counting fence. That morning five dead salmon parr and five dead suckers were in the down-trap. Ten dead salmon parr, 2½–3½ inches long, were found scattered among stones on the river bottom. In the morning the water temperature had fallen to –0.2 C from a low of 5.5 C on October 29; from October 23 to 28 it had ranged from 5.5 to 10.5 C. Elson (1967) records a similar observation on the Nashwaak River.

## DISCUSSION AND CONCLUSIONS

It is well known that aerial forest spraying of DDT to control insect pests is hazardous to fish and other aquatic fauna (Langford, 1949; Rudd and Genelly, 1956). Opinions on "safe" minimum rates of application based on experience have varied widely, as might be expected because of differences in methods of application, size and nature of watersheds, species susceptibility, and other factors. Observations associated with operational sprayings in New Brunswick, first in 1952 for application of DDT at 1 lb/acre, again in 1953 and 1954 for DDT at ½ lb/acre, showed that damage to young Atlantic salmon could be severe. They led in 1958 to the first attempts to find alternative methods of spruce budworm control that would cause less damage to aquatic fauna.

The simple caged-fish tests made in June 1954 inside and outside the operational spray area showed immediate effects of spraying on young salmon. Later that year the results were supported by the regular August–September population assessments of young wild salmon in the Northwest Miramichi area (Elson, 1967). Cage tests became standard procedure wherever it was necessary to check effects of operational sprayings or to compare different insecticides sprayed experimentally. Occasionally there were differences in survival rates of caged specimens: for example, after experimental spraying of DDT at ½ lb/acre in the Mactaquac area in 1959, salmon parr survived well for 3–4 weeks but many fry died quickly. It is suspected that a sufficient quantity of DDT did not reach the water to affect the larger specimens because the sprayed plots were very small and the streams were covered by a dense forest canopy. Usually, unexpected results in cage tests could be accounted for on the basis of cage position in relation to current or the physical condition of some groups of hatchery-produced specimens. Although from 1958 on, water samples were taken in the vicinity of cages for DDT determinations by the Chemical Control Section, Canada Department of Agriculture (later Forestry), data useful for interpreting caged fish mortalities are not available from these samples.

The caged specimens, and similar fish released above barrier fences, provided evidence that (*a*) DDT sprayed operationally at ½ lb/acre caused heavy loss of salmon parr within 3 weeks (1954); (*b*) DDT sprayed experimentally as a single application at ¼ lb/acre had no apparent short-term effects on salmon parr, but applications of DDT at 1 lb/acre and ½ lb/acre were followed by considerable losses; concurrent testing showed that DDD at ½ lb and ¼ lb/acre and malathion at ⅛ lb/acre were at least no more harmful to young salmon than DDT at ¼ lb/acre (1958, 1959); (*c*) in operational spraying, DDT at ¼ lb/acre was less harmful to young salmon and trout than DDT at ½ lb/acre (1960); (*d*) two operational sprayings of DDT at ¼ lb/acre about 10 days apart were as harmful as a single application of DDT at ½ lb/acre (1961 compared with 1960); (*e*) replacing Stearman with larger aircraft in operational spraying to cover square blocks, instead of irregular blocks usually bounded by streams, caused heavy fish losses even for single applications of DDT at ¼ lb/acre; the heavier losses may have occurred because many days were required to cover a spray block and spray could not be shut off over open water (1962); (*f*) experimental spraying of phosphamidon at 1 lb/acre had no apparent harmful effects on young salmon and trout (1962).

Observations at the counting fence at Curventon, 8 miles above tidehead and 10 miles below the downstream 1954 spray boundary on the Northwest Miramichi, showed many dead suckers and cyprinids. Suckers are known to be especially sensitive to DDT (Langford, 1949).

The reduction in frequency of up- and down-movements of brook trout and white suckers at the Curventon fence for several years starting in 1955 (Table VIII) was likely a result of DDT spraying. In the Miramichi area many trout appear to move from fresh to salt water and back, as do trout in Ellerslie Brook, P.E.I. (Smith and Saunders, 1958). At Curventon there are normally two peaks of upstream migratory activity of silvery-coloured trout weighing up to several pounds, namely a prominent early run in May and June and a fall run of only a few individuals. At peak times of upstream migration there are simultaneous downstream movements of non-silvery fish that have likely been long-time stream inhabitants. Possibly the 1954 spraying reduced the numbers of young trout in upriver areas that normally would have gone to sea and returned in 1955 and perhaps 1956. In 1956 and 1957 the upper Northwest Miramichi watershed was not sprayed and trout fry production was at least normal in 1955 (Keenleyside, 1959), as occurred for salmon (Elson, 1967). This could account for the large downstream trout migration in 1957.

The observations in autumn, at the onset of near-freezing water temperatures, of many dead young salmon in streams that had been sprayed with DDT that spring, led to speculation about the causes of delayed effects. In autumn 1960, laboratory experiments at the St. Andrews Station with salmon parr collected in an unsprayed stream and in a stream that had been sprayed that June with DDT at ½ lb/acre, included subjecting them to gradually lowering temperatures followed by continuous holding near 1 C. For a month there were no mortalities among the specimens from the unsprayed stream but over 80% of those from the sprayed stream died (Fisheries Research Board of Canada, 1961). Hence, DDT spraying can have important delayed effects on young salmon populations that could easily be overlooked.

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# Effects on Wild Young Salmon of Spraying DDT over New Brunswick Forests[1,2]

By P. F. Elson

*Fisheries Research Board of Canada*
*Biological Station, St. Andrews, N.B.*

## ABSTRACT

When DDT-in-oil was sprayed over large tracts of New Brunswick forest some of the spray got into drainage systems lying in the spray zones. As a result, many young salmon were killed and stocks were endangered.

Most New Brunswick salmon spend 3 years in rivers before migrating to sea as smolts. The young fish can conveniently be divided into three size-groups, which are roughly comparable to each year of age. The groups are: underyearlings, small parr (over 1 year old but 10 cm and under from tip of snout to tip of extended tail fin), and large parr.

Wild young salmon in unsprayed and sprayed streams were assessed for numbers in each size-group, abundance being expressed as population index = average number per 100 yards$^2$ of stream bottom.

*DDT at ½ lb/acre.* After such spraying young salmon of all size-groups were found in noticeably low numbers as compared with unsprayed situations. Underyearlings were only 2–10% as abundant (index reduced from 24 in places not affected by spray to less than 1 in most sprayed places), small parr only 30% (index reduced from 20 to 6), and large parr only 50% (index reduced from 12 to 6) as abundant.

*DDT at ¼ lb/acre.* After spraying at this dosage underyearlings were only about 50% as abundant as with no spraying; small parr were about 80% as abundant and large parr were affected hardly at all.

*DDT at ¼ lb/acre applied twice.* This was followed by low numbers of underyearlings, similar to those observed with a single application at ½ lb/acre. No equivalent data for parr are available but other information indicates effects similar to a single spraying with ½ lb DDT/acre.

*Downstream transport* of the DDT-in-oil mixture was an important factor in extending the harmful effects 30 or more miles below spray zones.

*Delayed mortality* followed 4 to 6 months after the early mortality described above. It was associated with the onset of winter cold. It may have removed a large proportion of parr which survived early mortality but quantitative measurements of this factor were not made on wild populations.

Reductions in numbers of young salmon were followed by declines in numbers of adults taken in fisheries and returning as spawners.

With no spraying in subsequent years, promptness of recovery of young salmon populations depended primarily on abundance of parent stock.

## INTRODUCTION

Aerial spraying of New Brunswick forests against the spruce budworm (*Choristoneura fumiferana* (Clem.)) began in 1952 (Webb et al., 1961). Kerswill (1967) outlined how this forest management technique impinged on the fauna of New Brunswick salmon streams. Figure 1 shows some of the well-known

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[2] The third in a series of papers on work of the Fisheries Research Board of Canada on effects of forest insecticides on salmon and aquatic insects.

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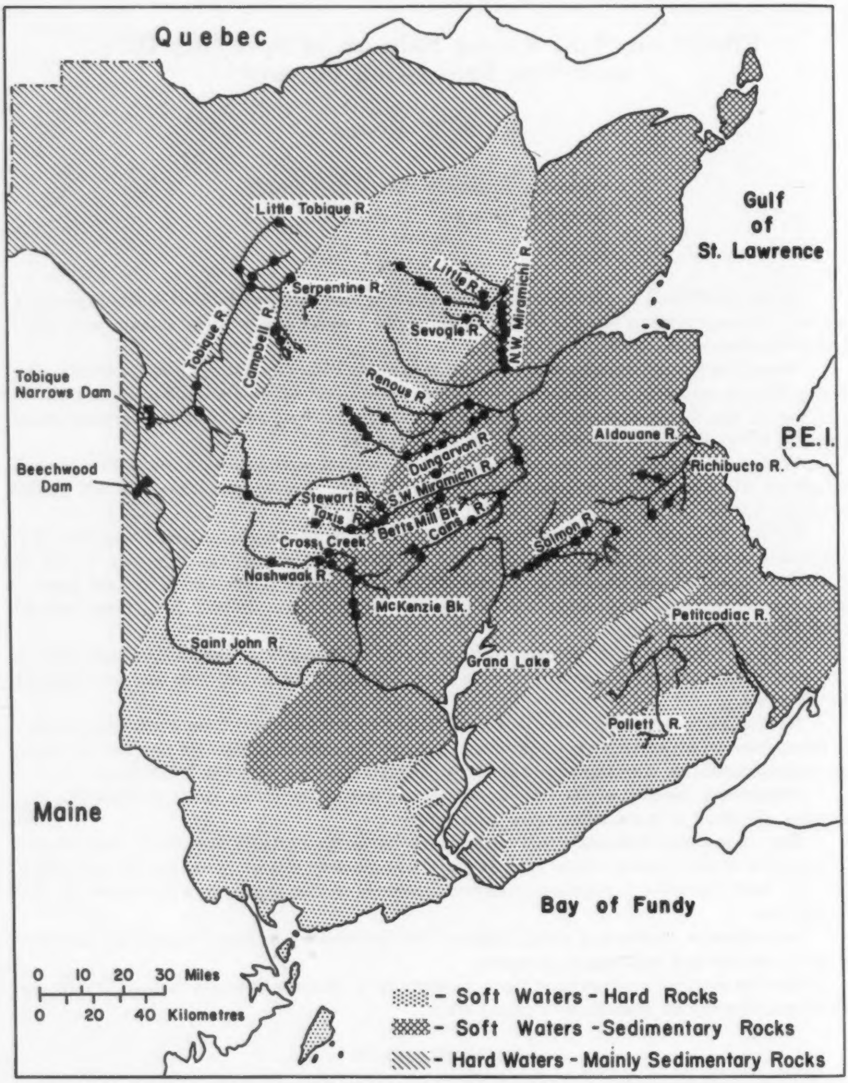
Quebec
Gulf of St. Lawrence
Little Tobique R.
Serpentine R.
Little R.
N.W. Miramichi R.
Sevogle R.
Tobique R.
Campbell R.
Tobique Narrows Dam
Renous R.
Beechwood Dam
Dungarvon R.
S.W. Miramichi R.
Stewart Bk.
Taxis R.
Betts Mill Bk.
Cains R.
Cross Creek
Nashwaak R.
Aldouane R.
Richibucto R.
P.E.I.
Salmon R.
McKenzie Bk.
Saint John R.
Grand Lake
Petitcodiac R.
Pollett R.
Maine
Bay of Fundy
0 10 20 30 Miles
0 20 40 Kilometres
░ - Soft Waters - Hard Rocks
▩ - Soft Waters - Sedimentary Rocks
▨ - Hard Waters - Mainly Sedimentary Rocks

FIG. 1. New Brunswick streams in which populations of wild young salmon were studied in relation to aerial forest spraying with DDT. Locations of all young salmon assessment stations used from 1951 to 1964 shown as black dots. Underlying geology adapted from Map 910A, Canada Department of Mines and Resources, 1949, and from Smith (1963) (adapted with permission of the copyright owners, the Regents of the University of Wisconsin, from David G. Frey [ed.] *In* Limnology in North America, The University of Wisconsin Press).

streams affected. Kerswill and Edwards (1967) also describe how such spraying resulted in immediate, often heavy mortality to young caged salmon. The present account deals with comparative abundance of wild young salmon observed in streams both before and after various types of spray treatment with DDT, up to and including the 1962 operation.

### Forest Spraying in New Brunswick

Productive forest land in New Brunswick covers 15,253,760 acres (1 acre = 0.405 ha) (Dominion Bureau of Statistics, 1964). This is 84% of the total area of the province. From a comparatively modest beginning of 186,000 acres in 1952, spraying of New Brunswick forests increased to a maximum of 5,691,000 acres (Fig. 2, 4, 5, 6, 8) in 1957. Only in 1959 was there no operational spraying.

In every year in which spraying was carried out, some part of one or more important New Brunswick salmon streams fell within the boundaries of sprayed areas. Many streams have been subjected to spraying in 2 or more years, sometimes in succession. Important salmon stream basins in the adjacent Gaspe Peninsula of Quebec, which provide some stock used in New Brunswick commercial fisheries (Belding and Préfontaine, 1938), have also been sprayed (Bergeron, 1959; Webb et al., 1961).

In 1952 (Fig. 2, *right*) the rate of application was 1 lb DDT/1 U.S. gal of solvent oil per acre (1.12 kg per 4.6 liters per hectare). From 1953 through 1958 the standard dosage was $\frac{1}{2}$ lb DDT/$\frac{1}{2}$ gal oil per acre (0.56 kg per 2.3 liters per hectare). Beginning in 1960 some operational spraying was done at $\frac{1}{4}$ lb DDT/$\frac{1}{2}$ gal per acre (0.28 kg per 2.3 liters per hectare).

### Atlantic Salmon Research Program in Relation to Forest Spraying

Kerswill (1967) has referred to the program of Atlantic salmon research established by the Fisheries Research Board of Canada on the Miramichi River in 1950. The first spraying of the Miramichi, in 1954, affected the Northwest Miramichi and Dungarvon Rivers, two streams involved in an experiment to test the value of merganser control (Elson, 1962a) for increasing adult salmon. The associated annual assessments of small fish in these two rivers indicated decreases in local populations of young salmon after spraying (Kerswill and Elson, 1955). Similar population assessments were then extended to other salmon streams.

### Life History and Ecology of Salmon Pertinent to Forest Spraying

Some knowledge of the Atlantic salmon's life history and ecology is essential to understanding how DDT forest spray, applied in late May and June, affects New Brunswick salmon. Jones (1959) gives a general account of Atlantic salmon. There are a few facts and terms which should be kept in mind throughout this account.

*Underyearling* salmon emerge from the spawning gravels in May and June. At first they live among the stones of the bottom (White, 1933; Hoar, 1937;

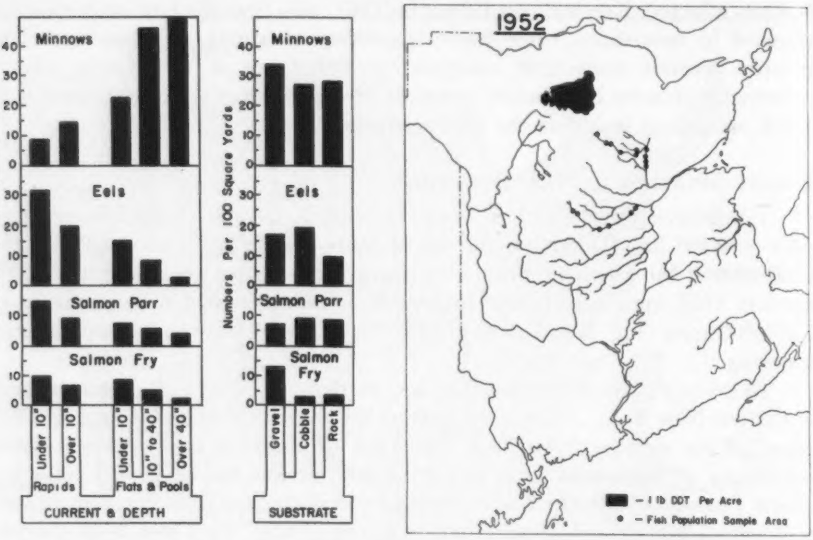

FIG. 2. (*Left*) Distributions of resident fish populations in respect to type of habitat. (Unpublished data from the Pollett River, N.B.) (*Right*) New Brunswick forest sprayed and young salmon assessment stations used in 1952.

Saunders, 1965). They eat small, aquatic stages of stream insects (White, 1936; Keenleyside, 1967). These habits make the young fish as well as their food vulnerable to DDT forest spray.

Later in their first summer and throughout most of their freshwater life the young salmon (called *parr* in their second and later years) tend to occupy home territories. They resist incursion by their brothers into these territories (Elson, 1942; Keenleyside and Yamamoto, 1962). Their homes are most frequently located in comparatively shallow riffles with gravel and cobble bottom (Elson, 1942; Fisheries Research Board of Canada, 1961a; Saunders and Gee, 1964). Figure 2, *left*, indicates, for the first time quantitatively, the relative importance of the various types of habitat. Parr eat food similar to that of underyearlings, but use larger organisms, and feed at the surface more than underyearlings. If either habitat or food is damaged, production of young salmon is reduced because many affected individuals fail to establish new homes elsewhere.

Freshwater life usually lasts 2 or 3 years. Most Maritime parr which attain a length of 10 cm (snout to tip of extended tail fin) by autumn become *smolts* the next spring (Elson, 1957a). As a rule smolts migrate seaward as water temperatures rise to 10 C and above (Elson, 1962a) in late May and June. A particular year-class could be exposed to three or four successive annual sprayings between emergence from the gravel and migration into the sea.

Many salmon mature after 1 year at sea, returning usually to their natal river as 2½- to 6-lb *grilse*. Others return after 2 or more years, weigh upwards from 7 lb, and are popularly just called ***salmon***.

Differences in duration of freshwater and sea stages result in adults of a year-class normally returning 4, 5, or 6 years after their parents spawned. Effects of unusual mortality, or survival, of the young in 1 year will thus be reflected in adult stocks in several later years.

### STREAMS STUDIED

The underlying geology of a region influences the mineral content, hence alkalinity, of its natural waters (Smith, 1963). DDT decomposes less rapidly under acidic than alkaline conditions (Ontario Department of Lands and Forests, 1949; Cope, 1965). Many New Brunswick salmon streams have low mineral content as indicated by electrical conductivity (Smith, 1962) and are acid (*p*H under 7.0). Smith's (1963) geological zonation of New Brunswick is incorporated in Fig. 1.

Surface geology affects the gradient and substrate of streams. New Brunswick highland areas are mostly hard igneous rock. The torrent-type streams have much round and loosely bedded gravel, cobble, and boulder (Ganong, 1903). The lowland areas are mainly softer sedimentary rock, with streams having more slack water and correspondingly less riffle. Even in rapids the flatter stones of the substrate are often firmly bedded in finer material.

Torrent-type streams provide more homes for young salmon (Fig. 2, *left*) than the slower streams, but the latter may produce more food per unit area of good young salmon habitat. To some extent these two factors appear to balance out in the streams studied, so that natural populations of young salmon are frequently of similar order in the two types. Actually, most better-known New Brunswick salmon streams have torrent-type upper reaches and comparatively slow-flowing lower reaches. Ganong (1902a, b, 1904, 1905, 1909, 1910, 1913) gives detailed descriptions of several.

Table I gives information on the streams studied: length, geology, electrical conductivity of summer low water, and importance for salmon angling.

## METHODS

### COLLECTING DATA

#### SELECTION OF AREAS FOR POPULATION ASSESSMENTS

The streams studied are comparatively inaccessible for much of their length. Areas in which to assess small fish were selected near points of reasonable access by ordinary light truck or 4-wheel drive vehicle. Most sampling areas covered the full width of the stream and were 20–30 yards (1 yard = 0.9 m) long so that operations could be completed in 1 day. Areas were selected to be representative of young salmon habitats in each stream. The same stations, with boundaries marked by blazes on trees or by similar landmarks, were used year after year.

TABLE I. New Brunswick streams in which population estimates of young salmon were made for studies on effects of forest spraying with DDT. (1 mile = 1.6 km; Ig = igneous; Sed = sedimentary; $K_{25}$ calculated as $K_{18} = \frac{10^6}{R_t\,[1 + 0.025\,(t-18)]}$ (from Smith, 1962) multiplied by 1.14 (from American Public Health Association, 1965)).

| River | Length, rock formation, and specific conductance ($K_{25}$) of water | | | | | | Salmon catch by angling 1949–59 | |
|---|---|---|---|---|---|---|---|---|
| | Upper reaches | | | Lower reaches | | | | |
| | Miles | Rocks | μmhos | Miles | Rocks | μmhos | Mean ± SD | Coefficient of variation (%) |
| Miramichi System | | | | | | | | |
| Main Southwest | 65 | Ig | 45 | 50 | Ig | 55 | 22,453 ± 7,659 | 34 |
| Northwest Miramichi | 15 | Ig | 45 | 50 | Sed | 60 | 3,689 ± 1,847 | 50 |
| Renous | 20 | Ig | 30 | 30 | Sed | 45 | 1,231 ± 544 | 44 |
| Dungarvon | 20 | Ig | 35 | 25 | Sed | 45 | 380 ± 231 | 61 |
| Cains | 35 | Sed | 30 | 15 | Sed | 60 | 1,653 ± 1,298 | 79 |
| Saint John System | | | | | | | | |
| Tobique | | | | | | | 1,121 ± 703 | 63 |
| Main | 30 | Sed | 60 | 25 | Sed | 105 | Included above | |
| Little Tobique | 20 | Sed | 60 | 10 | Sed | 180 | | |
| Mamozekel | 7 | Sed | 50 | 13 | Sed | 70 | | |
| Serpentine | 24 | Ig | 25 | 6 | Sed | 60 | | |
| Campbell | 22 | Ig | 35 | 18 | Sed | 35 | | |
| Nashwaak | 30 | Ig | 45 | 25 | Sed | 55 | [a]227 ± 200 | 88 |
| Salmon (into Grand Lake) | 30 | Sed | 80 | 15 | Sed | 80 | No record (very late run) | |
| Richibucto System | | | | | | | | |
| Molus | – | Sed | 50 | – | Sed | 90 | No record (very late run) | |
| Coal Branch | – | Sed | 220 | – | Sed | 225 | No record (very late run) | |
| West St. Nicholas | – | Sed | 75 | – | Sed | 100 | No record (very late run) | |

[a] Average for 1942 and 1952–59.

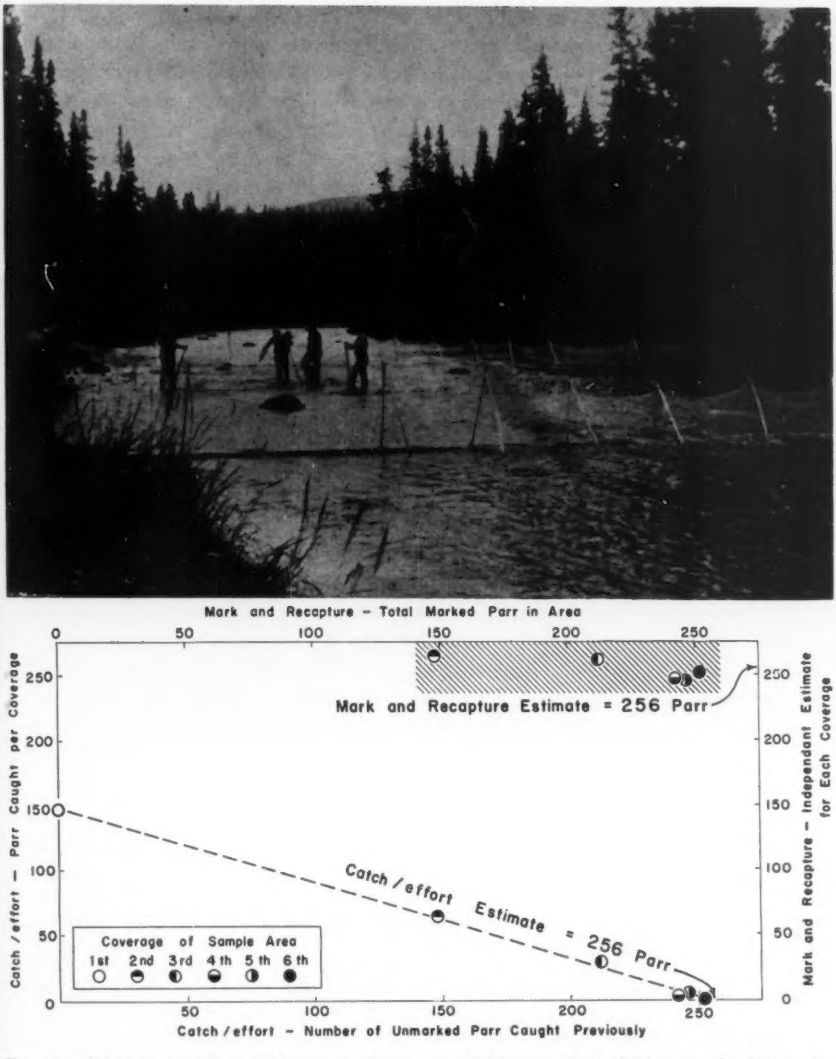

FIG. 3. (*Upper*) Sampling wild young salmon populations by electrofishing on the Serpentine River, tributary of the Tobique, September 21, 1961. (This is the upper station in Fig. 1.) (*Lower*) Development of mark-and-recapture and catch-effort estimates of parr population from the data obtained in the operation pictured above.

### APPARATUS

At the beginning of each assessment, a sample area was fenced off with barrier nets at its upper and lower boundaries. These nets, of ½-inch (1 inch = 2.5 cm) stretched mesh and with an 18-inch wide strip of burlap sewed along the footrope, were sealed to the bottom and raised above the surface of the water to act as barriers to fish moving into or out of the area (Fig. 3, *upper*).

In the early years fish were caught in a 15-ft seine set across the direction of stream flow. Two men drove fish into the seine (Elson, 1962a, p. 8). From 1953 on, practically all seining was accomplished with the aid of variable voltage d-c electrofishing generators; these had up to 500-v, 2- or 4-amp capacity, and were similar to that described by Smith and Elson (1950).

In soft waters (specific conductance under about 50 µmhos/centimeter) around 500 volts proved most effective; in harder waters (about 50–250 µmhos/centimeter) voltages down to about 300 v gave more effective fishing and minimized damage to fish. In 1955 the two-man block seine was improved by attaching an apron to its footrope. This apron seine, accessory dip nets, and their method of use are described by Elson (1962b).

### ASSESSMENT OF POPULATIONS

Estimates of the numbers of fish within a sample area were obtained by making several uniform coverages. In addition to salmon, some peckled trout, catostomids, cyprinids, and eels were taken; the additional species are not discussed in this report. Electrofishing was done back and forth across the area while proceeding from one barrier net to the other. About ¼ hr was needed to cover an area of 500 yards$^2$ (1 yard$^2$ = 0.836 m$^2$). The elapsed time required for the initial coverage or sweep was adhered to fairly closely for succeeding sweeps so that each would approximate a similar unit of effort. As a rule, six sweeps were made.

All fish taken in each sweep, except yearling and older salmon parr, were counted and removed to live-holding cages. The counts served as data for computing *unit-of-effort* ( = *catch/effort*) estimates of populations within an area, together with appropriate confidence limits (DeLury, 1951). After the first sweep, yearling and older parr were counted, the tip of one pelvic fin was removed, and the fish were returned to the area. After subsequent sweeps, unmarked and marked parr were tallied separately and all returned to the area after the unmarked parr had been finclipped with the same mark used after the first sweep. Data on successive captures of unmarked parr were used for catch/effort estimates. Data on captures of marked and unmarked parr were used for *mark-and-recapture* ( = *Schnabel* type) estimates with their appropriate confidence limits (DeLury, 1958).

High percentages of "catchable" parr were taken. Often about half those remaining to be captured were taken at each coverage. Six coverages frequently resulted in capture of 90–100% of the total parr estimated to be in an area.

Of the two methods used for calculating total parr within an area, Schnabel-type estimates appeared to be the more precise. In 80–90% of cases the actual catch was higher than the calculated lower (95%) confidence limits and thus provided a better lower limit. Using catch when applicable and calculated limits otherwise, lower limits averaged less than 5% below calculated estimates. In about 30% of cases, catch was slightly higher than its corresponding estimate and was then used as a measure of population. Upper (95%)

confidence limits were almost always available. They averaged less than 25% above estimates (or catch if this was higher than the corresponding estimate).

Catch/effort estimates for parr were usually slightly higher than their corresponding Schnabel estimates. They had wider (95%) limits, the lower often being about 15% below its estimate and the upper occasionally not even being available. Catch/effort estimates were, however, almost always contained within the 95% limits for Schnabel estimates. In general, estimates made by the two systems were similar (Fig. 3, lower) and Schnabel-type estimates are used generally in this report as measures of parr populations.

For populations of underyearling salmon (and of other species of fish), catch/effort estimates usually provided a useful index of abundance. Total catches for the several units of effort were occasionally higher than their corresponding estimates and were then used instead of the estimates. Catches were frequently higher than the calculated (95%) lower limits. Lower limits derived by calculation or from catch, whichever was higher, averaged less than 20% below their estimates. Upper (95%) limits were usually less than 50% above their corresponding estimates (or catches) but occasionally were much higher or even unavailable. In such cases, personal acquaintance with operations and specific areas frequently encouraged confidence in the usefulness of an estimate even though this could not be demonstrated by mathematical procedures.

Initial estimates of the parr population within an area included all sizes and ages together. But a sample of up to 50 specimens was taken from each area at the conclusion of the seining operation. The specimens were preserved in formalin and later examined for sizes and ages. Using the ratios found in this sample the estimated population was assigned as small (10 cm and under from tip of snout to tip of extended tail) or large (over 10 cm) parr, and to age-groups.

## TREATMENT OF DATA

To be representative of conditions across the width of streams, sample sections varied considerably in area. Not only was there variation between different areas, but particular areas sometimes varied from year to year because of differences in water level or because of minor changes in stream bed. For these reasons and to facilitate comparison between samples, results were reduced to a "population index" equal to the numbers of fish per 100 yards$^2$ (83.6 m$^2$) of stream bottom. (Multiply indices given by 1.2 to convert to values for 100 m$^2$.)

### VARIABILITY BETWEEN SAMPLES

Young salmon occur naturally in almost all Maritime stream systems not physically barred to upstream movement of adult fish. These streams offer a wide variety of habitat, varying from excellent to marginal. Considerable differences in local populations of wild young salmon are therefore to be expected. Over a period of years abundance of parent stock, as measured by commercial landings, can vary on a 5:1 ratio (Elson, 1957b). Local abundance of parent stock could influence local abundance of young, especially of underyearlings. During the period of this study the Department of Fisheries of Canada continued to plant upwards from about 5 million salmon fingerlings each year, many in the streams studied. Planting programs were, by agreement, maintained at their pre-spray level throughout and this contributed to variability between some local populations, depending on whether or not sampling stations were near a site used for hatchery liberations.

Within spray zones, population densities differed among samples taken near upstream boundaries, those well within spray zones, and those at varying distances below.

Variability between samples is indicated in the tables by the use of a standard error (SE) for each mean. (Means were derived without regard to confidence limits of individual estimates.) Means alone are used in most places throughout the text as providing a simple and meaningful measure of spray effects. Standard procedures for differentiating between means were used to assist in interpreting the data.

## RESULTS AND DISCUSSION

The scope of population sampling is indicated in Table II and, together with areas sprayed each year, in Fig. 1, 2, 4, 5, 6, and 9. A total of 62,516 young salmon were captured in about 5.7 acres (2.3 ha) of stream, much of which was sampled in successive years, to arrive at the 272 estimates of population made at 47 different stations. As an example of areas seined and numbers of young salmon caught in them over a period of years, totaled data for the three uppermost stations of the Northwest Miramichi from 1951 to 1962 are given in Table III.

TABLE II. Extent of assessment of young salmon populations, 1950–62.

| Stream | Period of sampling | No. of established sampling stations | Approx area (*yard*$^2$) sampled per year | Total samples | Young salmon caught: Underyearlings | Young salmon caught: Parr |
|---|---|---|---|---|---|---|
| Northwest Miramichi | 1950–62 | 10 | 7,000 | 112 | 15.126 | 21.193 |
| Dungarvon | 1952–56 | 6 | 6,000 | 27 | 5.308 | 3.590 |
| Renous | 1955–62 | 3 | 2,000 | 24 | 1.486 | 1.120 |
| Cains | 1955–62 | 5 | 3,500 | 39 | 5.568 | 2.933 |
| Southwest Miramichi | 1960–62 | 3 | 1,400 | 8 | 510 | 1.063 |
| Tobique | 1957–61 | 10 | 4,400 | 47 | 1.871 | 2.014 |
| Nashwaak | 1960–61 | 6 | 2,000 | 11 | 3 | 448 |
| Richibucto System | 1961 | 4 | 1,400 | 4 | 72 | 211 |
| Total |  | 47 | 27,700 | 272 | 29.944 | 32.572 |

To judge harm done to fish populations by spraying, some measurement of local populations either before or in the absence of spraying is needed. Populations not affected by spraying will be referred to as *normal* throughout this report.

Under spray conditions, depletion occurring between spraying in late May and June and annual censusing in late summer and autumn is considered "early mortality," to be described below. Kerswill and Edwards (1967) indicate that much mortality occurs within a comparatively short time after spraying. There may be additional loss through eating DDT-poisoned food or delayed-contact poisoning in the weeks following. Starvation in cold-water fish is a slow process,

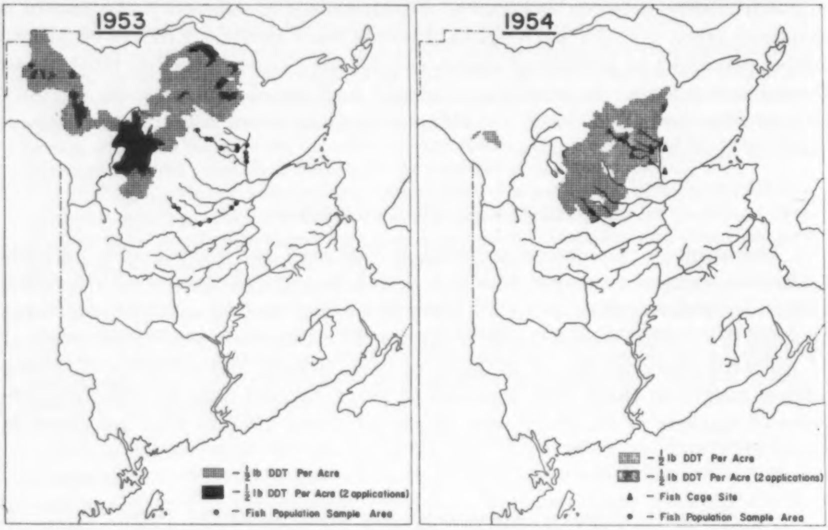
1953
▨ – ½ lb DDT Per Acre
■ – ½ lb DDT Per Acre (2 applications)
● – Fish Population Sample Area
1954
▨ – ½ lb DDT Per Acre
▨ – ½ lb DDT Per Acre (2 applications)
▲ – Fish Cage Site
● – Fish Population Sample Area

FIG. 4. New Brunswick forest sprayed and young salmon assessment stations used in 1953 and 1954.

TABLE III. Areas (yard$^2$) seined and numbers of young salmon (*fry* = underyearlings; *parr* = older) caught at the three upper sampling stations of the Northwest Miramichi River from 1951 to 1962.

| | Little Bald | | | Crawford | | | Forty-two | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Area | Fry | Parr | Area | Fry | Parr | Area | Fry | Parr | Area | Fry | Parr |
| 1951 | 546 | 39 | 169 | Not done | | | 1425 | 58 | 472 | 1971 | 97 | 641 |
| 1952 | 572 | 22 | 225 | 1150 | 221 | 671 | 1425 | 18 | 352 | 3147 | 261 | 1248 |
| 1953 | 616 | 56 | 314 | 1173 | 215 | 1029 | 1400 | 88 | 708 | 3189 | 359 | 2051 |
| 1954 | 616 | 0 | 196 | 1200 | 0 | 180 | 858 | 0 | 54 | 2674 | 0 | 430 |
| 1955 | 630 | 508 | 47 | 1350 | 826 | 26 | 825 | 609 | 21 | 2805 | 1943 | 94 |
| 1956 | 630 | 278 | 401 | 1350 | 85 | 903 | 825 | 70 | 490 | 2805 | 433 | 1794 |
| 1957 | 429 | 57 | 338 | 650 | 208 | 590 | 825 | 114 | 471 | 1904 | 379 | 1399 |
| 1958 | 462 | 17 | 146 | 783 | 17 | 403 | 675 | 31 | 261 | 1920 | 65 | 810 |
| 1959 | 462 | 78 | 87 | 783 | 499 | 322 | 650 | 22 | 243 | 1895 | 599 | 652 |
| 1960 | 429 | 127 | 120 | 702 | 150 | 401 | 675 | 146 | 256 | 1806 | 423 | 777 |
| 1961 | 462 | 6 | 172 | 729 | 189 | 394 | 650 | 66 | 301 | 1841 | 261 | 867 |
| 1962 | 495 | 185 | 45 | 783 | 155 | 120 | 675 | 133 | 70 | 1953 | 473 | 235 |

an unlikely cause of death until emaciation is extreme. Dead and moribund fish picked up in sprayed areas 4 months after spraying, although thin, were seldom extremely emaciated. Large losses in populations were evident within 1½ months after spraying, likely because the effects were more direct and rapid than would be possible through starvation.

## NORMAL ABUNDANCE

### UNDERYEARLINGS

For the normal unsprayed condition a population index of 24 underyearlings per 100 yards$^2$ was selected as a reasonable average value.

Indices of normal abundance of underyearling salmon calculated on several bases are given in Table IV, column 1, the index in the second-last row being an average value. For indices listed for individual streams and for "All Miramichi samples," all samples for years in which no spray was applied were used whether or not these areas had been sprayed in previous years. Some indices for unsprayed stations in 1955 are included. The year 1955 (see "Recovery After Spraying") was a year of particularly abundant salmon underyearlings in a number of Maritime streams, whether or not they had been sprayed earlier. Therefore, for the Miramichi, two other means for the normal condition are included in Table IV. These two means are not distinguishable from each other at any reasonable level of probability by standard statistical tests, or from the "All samples" index appearing in the second-last row of Table IV.

### SMALL PARR

The normal index is 20 small parr per 100 yards$^2$ of stream bottom.

Data on abundance of small parr are summarized in respect to spray history in Table V. The Tobique samples for unsprayed areas (column 1), taken in 1959, 1960, and 1961, represent spawning from 1957 to 1959. Adult stocks counted through the Tobique Narrows fishway, operated by the Department of Fisheries of Canada at the mouth of the river, were then comparatively low (Fig. 11). By comparison with other streams (Elson, 1957c), the Tobique was underseeded in those years. The "All samples" index (Table V) for unaffected populations of small parr (18.0 per 100 yards$^2$) is biased by this low value. For the purpose of generalization an index of 20 was chosen. This falls about midway between the lower 95% confidence limits of the "All Miramichi samples" value and the upper limits of the "All samples" value.

### LARGE PARR

The normal index for large parr is 12 per 100 yards$^2$ (Table VI).

## DDT AT ½ LB/ACRE

There is a conspicuous contrast between means for samples of young salmon having no history of spraying and those for samples affected by spraying at ½ lb DDT/acre (Tables IV–VI).

### UNDERYEARLINGS

In the first year (1954) of spraying on the Northwest Miramichi and Dungarvon Rivers, a total of only three underyearling salmon were caught in the 10 stations examined. The year before, 1528 underyearlings were taken from nine of these stations.

TABLE IV. Abundance of late-summer Atlantic salmon underyearlings in the *same* sample areas of New Brunswick streams when areas were *not* subjected to spray and when they were affected by spray at ½ lb DDT/½ U.S. gal of oil per acre in year of sampling, expressed as indices ( = mean numbers found per 100 yards$^2$) and their standard errors (SE = s/ $\sqrt{N}$) for all samples receiving similar treatment.

| | (1) No spray in year of sampling | | (2) Spray in year of sampling | | (3) Downstream drift of insecticide in year of sampling | |
|---|---|---|---|---|---|---|
| Stream | Samples | Index | Samples | Index | Samples | Index |
| Miramichi system | | | | | | |
| Northwest Miramichi | 66 | 29.2±3.1 | 14 | 0.6±0.2 | 7 | 4.1±2.8 |
| Dungarvon | 21 | 29.4±6.0 | 5 | 1.1±0.9 | 1 | 0.8 – |
| Renous | 7 | 18.2±4.1 | 6 | 0.2±0.2 | 2 | 22.4[c]±12.0 |
| Cains | 3 | 29.6±6.2 | 2 | 19.3±4.1 | 0 | – – |
| (1) All Miramichi samples | 97 | 28.4±2.5 | 27 | 2.0±1.0 | 10 | 7.4[c]±3.6 |
| (2) Excluding 1955 fry | 77 | 23.7±2.4 | | | | |
| (3) Before any spraying | 59 | 24.1±2.4 | | | | |
| (4) Excluding samples near upstream spray boundaries[a] | | | 25 | 0.6±0.2 | | |
| Saint John system | | | | | | |
| Tobique | | | | | | |
| (1) All samples | 32 | 11.4±3.1 | 12 | 5.5±4.6 | 1 | 0.5 – |
| (2) Excluding one sample with known recent hatchery stocking | – | – – | 11 | 0.8[b]±0.9 | | |
| Nashwaak | 0 | – – | 5 | 0.1±0.1 | 0 | – – |
| All streams | | | | | | |
| (1) All samples | 129 | 24.2±2.1 | 44 | 2.7±1.4 | 11 | 6.8±3.4 |
| (2) Excluding samples with known bias | – | – – | 41 | 0.6±0.3 | | |

[a] The lowest Cains River station was only 2 miles inside the upstream spray boundary on this stream in 1956 and 1957.

[b] One station in this group was later learned to be a regular hatchery-planting site, but by then it could not be confirmed that planting had been carried out before assessment in the year of spraying. This sample gave an index of 10.1 underyearlings; the remaining 10 samples had no underyearlings at all.

[c] Numbers may have included survivors of some post-spray plantings of hatchery fish.

In general, underyearlings within the zone of spray were sparse indeed (Table IV, column 2). The "All samples" index (2.7) shows reduction by about 90%. But this mean includes three stations which received different treatment from the other stations, as described below.

Excluding the three atypical samples, the mean index is 0.6 underyearlings. This, compared to a normal index of 24, implies 98% reduction of underyearling salmon living well within upstream boundaries of spray zones.

TABLE V. Abundance of small Atlantic salmon parr (up to 10 cm from tip of snout to tip of extended tail; mostly yearlings) in the *same* sample areas of New Brunswick salmon streams when areas were *not* subjected to spray and when they were affected by spray at ½ lb DDT/½ U.S. gal of oil per acre in year of sampling, expressed as indices ( = mean numbers found per 100 yards²) and their standard errors (SE = s/ $\sqrt{N}$) for all samples receiving similar treatment.

| | (1) No spray at any time | | (2) Spray in year of sampling only | | (3) Spray one year before only | | (4) Spray or drift year before and year of sampling | |
|---|---|---|---|---|---|---|---|---|
| Stream | Samples | Index | Samples | Index | Samples | Index | Samples | Index |
| Northwest Miramichi | 63 | 23.9±2.1 | 10 | 6.6±2.2 | 14 | 0.8±0.3 | 7[a] | 5.5±1.4 |
| Dungarvon | 15 | 16.5±2.0 | 5 | 3.9±0.6 | 2 | 0.0±0.0 | 0 | – – |
| Renous | 0 | – – | 3 | 6.3±3.6 | 0 | – – | 3[b] | 0.2±0.2 |
| Cains | 0 | – – | 1 | 5.3± – | 1 | 3.7 – | 1[c] | 6.4 – |
| All Miramichi samples | 78 | 22.5±1.8 | 19 | 5.8±1.2 | 17 | 0.9±0.3 | 11 | 4.1±1.2 |
| Tobique | 21 | 3.6±1.5 | 3 | 11.2±5.8 | 7 | 0.0±0.0 | 10[d] | 1.7±0.8 |
| Nashwaak | 0 | – – | 5 | 10.6±6.4 | 0 | – – | 0 | – – |
| All streams | 99 | 18.0±1.6 | 27 | 7.3±1.5 | 24 | 0.6±0.2 | 21 | 3.0±0.7 |

[a] Spray as fry and drift as parr, 4 samples; drift as fry and spray as parr, 3 samples.
[b] Spray as fry and drift as parr, 2 samples; spray as fry and spray as parr, 1 sample.
[c] Spray as fry and spray as parr, 1 sample.
[d] Spray as fry and spray as parr, 9 samples; drift as fry and spray as parr, 1 sample.

TABLE VI. Abundance of large Atlantic salmon parr (over 10 cm from tip of snout to tip of extended tail) in the *same* sample areas of New Brunswick streams when areas were *not* subjected to spray and when they were affected by spray at ½ lb DDT/½ U.S. gal of oil per acre in year of sampling, expressed as indices ( = mean numbers found per 100 yards$^2$) and their standard errors (SE $= s/\sqrt{N}$) for all samples receiving similar treatment.

| | (1) Not sprayed at any time | | (2) Spray in year of sampling only | | (3) Spray year before only | | (4) Spray 2 years before only | | (5) Spray of drift year before and year of sampling | | (6) Spray 2 years before and drift or spray year of sampling | | (7) Spray or drift each of last 3 years | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stream | Samples | Index | Samples | Index | Samples | Index | Samples | Index | Samples | Index | Samples | Index | Samples | Index |
| Northwest Miramichi | 53 | 13.5±2.0 | 7 | 7.3±1.8 | 0 | – | 3 | 2.1±1.0 | 6 | 5.4±1.6 | 7 | 0.3±0.1 | 1 | 2.4 |
| Dungarvon | 12 | 5.7±1.7 | 3 | 3.6±0.2 | 6 | 3.7±0.8 | 3 | 3.1±1.2 | 0 | – | 3 | 2.7±1.0 | 0 | – |
| Renous | 0 | – | 0 | – | 3 | 2.9±0.5 | 1 | 1.7 | 3 | 3.2±1.6 | 3 | 1.7±0.6 | 2 | 1.0±0.4 |
| Cains | 1 | 4.4 | 1 | 3.5 | 0 | – | 1 | 1.5 | 1 | 3.6 | 0 | – | 0 | – |
| All Miramichi samples | 66 | 12.0±1.2 | 11 | 6.0±1.3 | 9 | 3.4±0.5 | 8 | 2.4±0.6 | 10 | 4.6±1.0 | 13 | 1.1±0.4 | 3 | 1.5±0.5 |
| Tobique | 14 | 9.4±2.2 | 1 | 18.0 | – | – | 10 | 6.7±1.9 | 3 | 11.2±1.3 | 2 | 2.3±1.9 | 7 | 4.3±1.4 |
| Nashwaak | 0 | – | 5 | 4.7±1.8 | 0 | – | 0 | 0 | 0 | – | 5 | 4.5±1.9 | 0 | – |
| All streams | 80 | 11.5±1.2 | 17 | 6.3±1.2 | 9 | 3.4±0.5 | 18 | 4.8±1.1 | 13 | 6.1±1.2 | 20 | 2.1±0.6 | 10 | 3.4±1.0 |

Among Cains River samples, the sampling station nearest the mouth of the stream, alone, received ½ lb DDT/acre in 1956 and 1957. The zone of spray extended only 2 miles upriver from the station (Fig. 5, 6). Underyearlings were about two-thirds as abundant as in years without spray, and much more abundant than in stations farther inside zones of direct deposition. With these two Cains samples included, the mean index for the Miramichi system is 2.0 (Table IV). With these two samples excluded, the mean index is 0.6.

For the Tobique system, data for underyearlings sprayed in year of sampling are biased by a substantial postspray hatchery planting on one area. Excluding this sample, the Tobique mean index for sprayed underyearlings is 0.8.

### SMALL PARR

For small parr sprayed in the year of sampling the generalized index is 6. As compared with the normal index of 20, an index of 6 suggests that ½ lb DDT/acre spraying causes early mortality of 70% of young salmon between about 7 and 10 cm long.

Among samples of small parr sprayed in the year of sampling (Table V), indices for the Tobique and Nashwaak diverged from all others, largely caused by a single sample in each stream. To obtain a generalized index, a value was selected midway in the range of overlap between the 95% confidence limits for the "All Miramichi samples" and the "All samples" estimates, as was done for the normal small parr index.

### LARGE PARR

The mean index for large parr (Table VI) which were sprayed only during their final spring is 6. The difference between this and the normal index of 12 indicates a 50% reduction.

## DDT AT ¼ LB/ACRE — GENERAL

Because of the serious effects which ½ lb DDT/acre forest spraying had on local stocks of young salmon, efforts were made in cooperation with the forest entomologists to find a dosage giving useful budworm control with less damage to fish. As a result of studies on the Richibucto system in 1958 and the Saint John in 1959 (Kerswill, 1967), DDT at ¼ lb/acre was selected for large-scale trial.

Approximately half of the 2.6 million acres sprayed in 1960 were treated with this lighter dosage (Fig. 9). Several established young salmon sampling stations were within the zone of lighter application. Of the five stations on the Cains River examined annually since 1955, four never sprayed before received the lighter dose.

In 1961 some areas received two applications, both at ¼ lb DDT/acre, spaced a week or two apart. In 1962 the entire Cains received such respray treatment. The pattern of spray application was such that the surrounding forest received two applications, while downstream reaches of the river received some DDT on 11 different days by either direct spraying or water transport from above (Kerswill and Edwards, 1967).

There are fewer data for abundance of young salmon in areas receiving ¼ lb/acre spray, either once or twice than for ½ lb/acre spray. This is partly

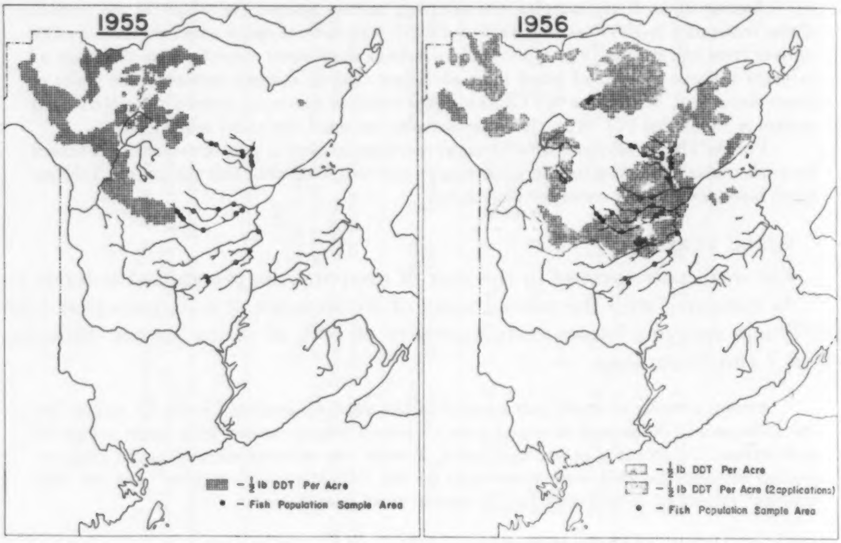
1955
▨ – ½ lb DDT Per Acre
• – Fish Population Sample Area
1956
▨ – ½ lb DDT Per Acre
▨ – ½ lb DDT Per Acre (2 applications)
• – Fish Population Sample Area

FIG. 5. New Brunswick forest sprayed and young salmon assessment stations used in 1955 and 1956.

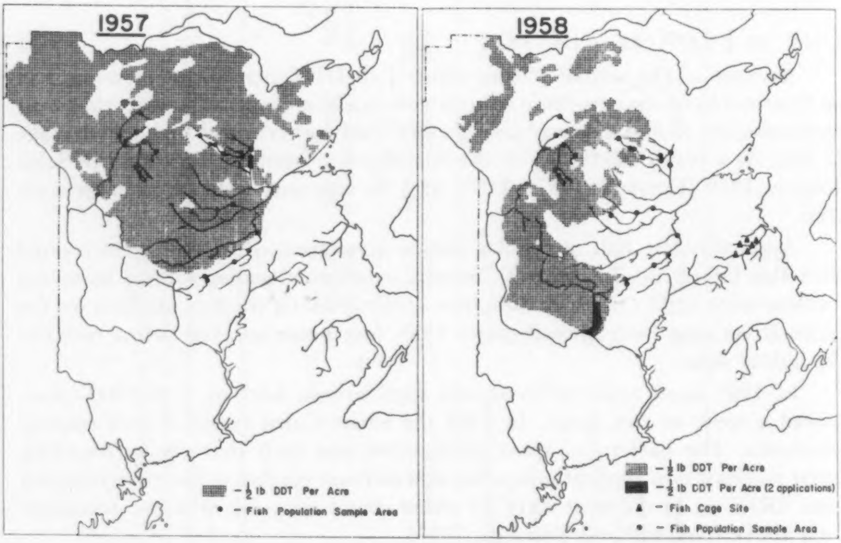
1957
▨ – ½ lb DDT Per Acre
• – Fish Population Sample Area
1958
▨ – ½ lb DDT Per Acre
■ – ½ lb DDT Per Acre (2 applications)
▲ – Fish Cage Site
• – Fish Population Sample Area

FIG. 6. New Brunswick forest sprayed and young salmon assessment stations used in 1957 and 1958.

because most of the sampling stations receiving the lighter dosage already had a history of spraying within the freshwater life of the older young salmon present.

Much of the data (Table VII) comes from the Cains River. Despite comparatively normal seeding with underyearlings, the indices for small and large parr in this stream, even before spraying, were comparatively low (compare "No spray" data in Table VII, A and B).

Data from the Richibucto system in 1960 (Table VII) are presented separately partly because the preceding autumn, with exceptionally low water, was reputed to be unusually poor for local runs of adult salmon into these small streams. In addition, this system exhibits somewhat different water quality from the other systems (see "Streams Studied" and Table I). Here, as in the other areas, the assessments indicate depression of young salmon abundance by this kind of spraying.

TABLE VII. Abundance of young Atlantic salmon in the *same* sample areas of streams when areas were not subjected to spray and when they were subjected to one or two applications at ¼ lb DDT/½ U.S. gal oil per acre. Abundance given as indices ( = mean numbers/100 yards$^2$) and their standard errors (SE = $s/\sqrt{N}$) for all samples receiving similar treatment; number of samples in parentheses under each mean. Figures in upper row of A and B from column 1, "All streams," of Tables IV, V, and VI.

| | Underyearlings | Small parr | Large parr |
|---|---|---|---|
| A. All data except from Richibucto system | | | |
| No spray | 24.2±2.1 | 18.0±1.6 | 11.5±1.2 |
| | (129) | (99) | (80) |
| One application | 11.3±3.8 | 16.9±5.1 | 7.9[a]±3.0 |
| | (15) | (6) | (7) |
| Two applications | 3.8±2.1 | – | – |
| | (10) | (0) | (0) |
| B. Data from Cains River only | | | |
| No spray | 25.2[b]±3.8 | 9.4±1.6 | 5.2±0.6 |
| | (26) | (22) | (21) |
| One application | 9.9±3.9 | 6.9[c]±1.9 | 3.5±1.2 |
| | (6) | (5) | (5) |
| Two applications | 0 to several[d] | – | – |
| | (3) | (0) | (0) |
| C. Richibucto streams in 1960 | | | |
| No spray | 5.6 | 14.8 | 5.8 |
| | (2) | (2) | (2) |
| One application | 1.8 | 10.5 | 2.1 |
| | (2) | (2) | (2) |

[a] Includes two stations on Main Southwest Miramichi in 1960, sprayed at ½ lb/acre in 1958 but with moderately good populations of large parr.

[b] Excludes lowermost station, affected by ½ lb/acre spraying, in 1956 and 1957.

[c] Excludes North Branch station, which had no fry in 1959, the 1958 spawners having been barred by a beaver dam.

[d] See text under heading "DDT at ¼ lb/Acre — General."

## DDT AT $\frac{1}{4}$ LB/ACRE — SINGLE APPLICATION

### UNDERYEARLINGS

Whereas the observed mean population index for underyearling salmon in unsprayed situations is about 24, the mean for samples exposed to a single application of $\frac{1}{4}$ lb DDT/acre is about half this value. Therefore, a 50% loss of underyearlings is attributed to this kind of spraying.

### SMALL AND LARGE PARR

For small parr, indices were slightly lower than in unsprayed situations (Table VII, A and B) suggesting that between 6 and 27% were killed. Using the normal index of 20 and accepting the value in Table VII, part A, one application of $\frac{1}{4}$ lb DDT/acre reduced small parr to an index of about 17, representing a 15% loss.

For large parr, Table VII, A and B, shows a reduction of over 30% for a single application. DDT applied at $\frac{1}{2}$ lb/acre appeared to kill more small than large parr (70% as against 50%). It seems unlikely that this order would be reversed with the lighter dosage, and there are fewer samples. Early mortality of large parr from this spraying should be regarded as negligible.

It seems a reasonable summation to say that spraying DDT at $\frac{1}{4}$ lb/acre results in 15–20% fewer small and large parr combined, but the reduction may sometimes be less than this. It seems unlikely that such damage, inflicted in occasional years and only once on any year-class, could be distinguished in resulting stocks of adult salmon from natural causes of variation (Elson, 1957b; 1962c).

## DDT AT $\frac{1}{4}$ LB/ACRE — TWO APPLICATIONS

Among the 10 samples on young salmon abundance after two applications at $\frac{1}{4}$ lb DDT/acre (Table VII), one had received a substantial post-spray planting of hatchery underyearlings. Another was within 1 mile of the upstream boundary of the respray treatment. It, too, had a good underyearling population. Among the eight samples taken well within the zone of application of the first and second sprays, no underyearlings were found in six stations and the indices in the other two were 1.1 and 0.4. The reduction appears to have been essentially the same as for a single application at $\frac{1}{2}$ lb/acre, i.e. from 90 to 98%.

Scarcity of parr attributable to earlier spraying precluded opportunity to observe normal populations of older fish given re-spray treatment. Small parr on the Cains in 1962 received such treatment and little or no spray as underyearlings in 1961. But as underyearlings they were sparse after low water and consequent deficient spawning in the autumn of 1960. Studies on caged fish given re-spray dosage (Kerswill and Edwards, 1967) indicate that mortality to parr is heavier than with a single application of $\frac{1}{4}$ lb/acre and approaches that accompanying $\frac{1}{2}$ lb/acre spray.

## DOWNSTREAM EFFECT

Forest spraying with DDT-in-oil affects not only fish living within the zone of spraying, but those living some distance downstream. Data are available only from $\frac{1}{2}$ lb DDT/acre sprayings.

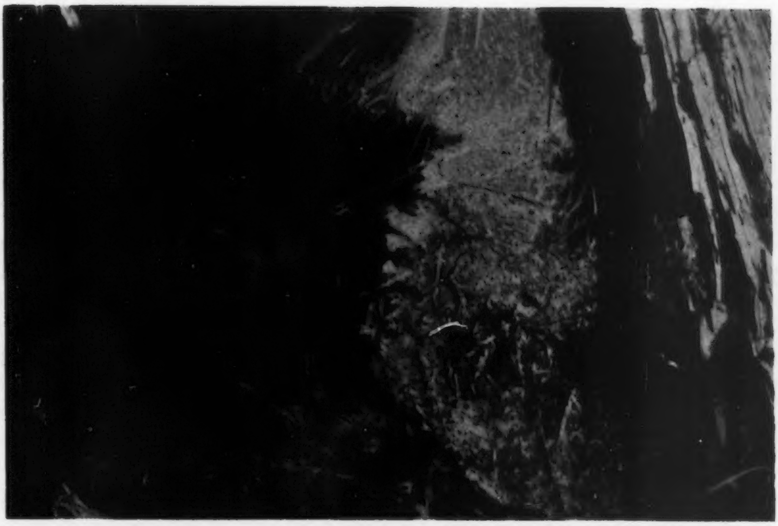

FIG. 7. Red-dyed DDT-in-oil solution, after accidental spillage of spray, shows how the insecticide was transported downstream, with some coming to rest on the stream bottom along the way. Similar, though less spectacular, examples were seen when spray was applied operationally. Direction of flow is from upper left and upper centre towards lower right hand corner of picture. Note in middle of picture submerged blobs of oil adhering to bottom material. (Photo taken on the Mactaquac River, 1959, by Dr C. J. Kerswill.)

Elson — J. Fish. Res. Bd. Canada.

Downstream transport of poison was well demonstrated during experimental spraying operations over the Aldouane River in 1958 and the Mactaquac River in 1959. The solvent oil had been dyed a bright red. Spray falling on the water agglomerated into red strings and rafts of oil which drifted on the water surface, holding up against obstructions and accumulating in quiet eddies. As the floating masses of oil entered more turbulent reaches, little gobs broke away and were churned to the bottom, where they clung to submerged vegetation or other substrate material (Fig. 7). Because of prime interest in the spray zone, little attempt was made to discover the extent of this downstream transport, but Dr C. J. Kerswill (personal communication) said that coloured droplets could be seen on the bottom at least ½ mile below the downstream spray boundary on the Mactaquac. The degree and distance of the downstream effect would vary with nature of the water flow. Much turbulence, as opposed to smooth-flowing water, favours faster deposition on the bottom and the accompanying mixing in the water makes the DDT more deadly to young salmon (Gagnon, 1958); inflowing unsprayed water accelerates dilution.

Table IV, column 3, gives population indices for underyearlings downstream from spray zones. Data on parr are insufficient to show any order beyond a general decrease in numbers below spray zones. The scarcity of fish reflects contamination by downstream drift of DDT-in-oil applied upstream. This transport by flowing water is not to be confused with aerial drift during spraying operations, which also occurs. For example, Bridges and Andrews (1961), in Montana, found 0.01 ppm of DDT in stream water taken ½ hr after spraying "although the plane didn't spray within one-quarter mile of the stream." Contamination of salmon-rearing grounds by aerial drift was not included in the studies reported here.

The data for underyearlings show that mortality can occur a considerable distance below spray zones. To about 5 miles downstream from spray boundaries (Fig. 8), population indices were as low as within spray zones. In a case (Little River in 1958, see Fig. 1, 6 and 8) where spray was applied on, or around, about 500 acres of headwater lakes but scarcely at all on the stream below, no underyearlings were found 12 miles downstream; the average index at this station for 6 years without spray was 57. In two samples, 10 and 15 miles downstream respectively, there were normal indices (Fig. 8, samples 9 and 10). The two streams involved had received substantial contributions of unsprayed water above the sampling stations. Also, both stations were near distribution points for hatchery-reared underyearlings. The appearance of underyearling specimens collected in these two assessments gave rise to suspicion that the indices were affected by recent hatchery plantings, though this could not be confirmed.

Reduction of underyearling populations to 60 or 70% of normal within 2 miles of upstream spray boundaries (see "DDT at ½ lb/Acre; Underyearlings") and reduction to 5% or less some miles below downstream spray boundaries indicate that much of the damaging effect comes from the DDT-in-oil mixture

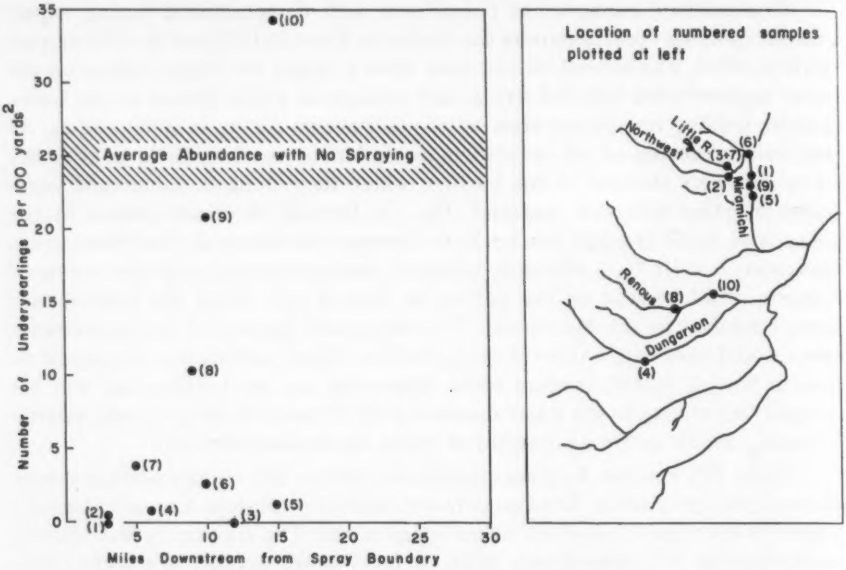

FIG. 8. Numbers of underyearling salmon found at various distances below downstream spray boundaries. Locations of samples indicated in map on right. The samples can be referred to the appropriate spray maps as follows: No. 4 on Fig. 4, 1954; No. 2, 6, 7 on Fig. 5, 1956; No. 1, 5, 9 on Fig. 6, 1957; No. 3, 8, 10 on Fig. 6, 1958. No. 8, 9, and 10 are at or near points commonly used for liberation of hatchery-reared underyearling salmon (see text under heading "Downstream Effect").

being carried downstream. The low solubility of DDT in water may actually enhance the chance of its effective spread without effective dilution. Fish may make contact with the poison by rising to the surface through it, by foraging over the bottom and touching the mixture adhering to the substrate, or by eating insects which have been poisoned either at the surface or on the substrate. It is probable that freshets during or soon after spraying would facilitate discharge of DDT by speeding and altering flow patterns, as well as by increasing dilution.

Cope (1961), reporting on DDT (1 lb/acre) used against the spruce budworm on the Yellowstone River, found, by chemical analysis, DDT 55 miles downstream from the sprayed area. Although no mortality to fish was observed, trout 85 miles downstream contained traces of DDT. Fish taken 2 years after spraying contained DDT in sublethal amounts. This suggests the possibility that dangerous levels may build up in young salmon well below sprayed areas if there should be repeated annual sprayings on one year-class.

The available data on populations downstream from the zone of spray can be summarized broadly as follows. (1) Underyearlings are commonly reduced to almost as low numbers in the 5 miles of stream immediately below as within sprayed areas. Similar reduction can occur to at least 15 miles, perhaps farther, downstream. (2) At about 10 miles downstream, populations may be very low to normal, averaging about half normal density. (3) It seems possible that some effect may be exerted to 20 miles downstream, or farther. (4) The more unsprayed influent water enters below, the more rapidly fish populations approach normal, presumably a dilution effect.

## DELAYED MORTALITY

The work of Bridges et al. (1963) on disappearance of DDT in a farm pond, and of Allison et al. (1964) on effects of sublethal doses of DDT on cutthroat trout, supports the belief that most of the loss of young Atlantic salmon reported here is properly attributed to effects occurring soon after spraying. Bridges and his co-workers (1963) found that DDT in detectable amounts had disappeared from a hard-water pond by 3 weeks after treatment. Disappearance may be slower (Ontario Department of Lands and Forests, 1949; Cope, 1965) in the soft New Brunswick waters (Leverin, 1947) under consideration. However, hatchery-reared underyearling salmon planted in sprayed waters a month or two after spraying have survived for a month or more without unusual losses, although with the scarcity of sufficient food insects they were thin (unpublished data). Warner and Fenderson (1962) found that mortality of stream trout in DDT-sprayed (1 lb/acre) Maine forests occurred largely within a 2-week period after spraying.

Although the present account is concerned largely with early mortality, delayed mortality may be an important factor in reducing salmon production.

Kerswill (1967) reports that in streams sprayed with ½ lb DDT/acre dead parr have frequently been seen with the onset of cold temperatures in late autumn. Normally, dead wild parr are rarely seen in a stream. In early October 1960 with the stream temperature falling below 10 C (50 F) I picked up 13 dead parr along 200 yards of the 150-ft wide Nashwaak River, sprayed with ½ lb DDT/acre 4 months earlier. Judging by the state of preservation, all had died recently.

The "All streams" index (0.6) for small parr sprayed as underyearlings, including those near the upstream limit of spray zones (Table V, column 3), is materially lower than the "All samples" index (2.7) for underyearlings a few months after spraying (column 2 of Table IV). This suggests up to about 80% mortality of the underyearlings in the intervening year. Again, comparing the index (3.4) for large parr sprayed 1 year before (Table VI, column 3) with the index (7.3) for small parr in the year of spraying (Table V, column 2) indicated over-winter mortality was slightly over 50%. Survival rates for young New Brunswick salmon during their freshwater life have been reported as between about 40 and 60% per year (Elson, 1962c). Other data (Elson, 1962a, Table XXV) suggest that under normal circumstances high year-to-year survival rates are associated with sparse populations. In the instances above, survival rates were only low to moderate with sparse populations, suggesting the possibility of more than usual mortality of the sprayed fish.

Ogilvie and Anderson (1965) show that exposure to DDT adversely alters temperature responses of young Atlantic salmon. Laboratory observations have confirmed that DDT-sprayed wild parr are more likely to die in cold water (5 C and below) than are unsprayed parr (Fisheries Research Board of Canada, 1961b). DDT is more toxic to some aquatic forms at low temperatures than at high (Sanders and Cope, 1966). Whether cold-weather mortality of sprayed young salmon results from increased toxicity of a formerly sublethal dose, from starvation-induced liberation of DDT stored in their fat (Rudd, 1964), from addition of stresses (Hoar, 1966), or from some other mechanism, is not known.

For the present, it must suffice to point out that in addition to the early mortality soon after the application of DDT spray, there are delayed adverse effects on young salmon survival.

## Spraying on One Year-Class in 2 or More Years

Reference to the maps showing areas sprayed in different years (Fig. 2, 4, 5, 6, 9) shows that many areas were sprayed in consecutive years or re-sprayed after a lapse of only 1 year. Repeated spraying may impinge two or three times on the same year-classes of salmon. Numbers of parr found at stations which received repeated application, whether directly or via downstream water transport (column 4 of Table V and columns 5, 6, and 7 of Table VI) were, on the whole, materially less than when parr received a single application. In general, the population indices indicate that the final result of repetitive treatment was the sum of the effects of each spraying on particular size-groups.

## Recovery after Spraying

### DATA PERTINENT TO RECOVERY

Tables VIII, IX, and X summarize available data on the recovery of salmon production after application of ½ lb DDT per/acre.

Data on post-spray year-classes of young salmon were first gathered in 1955. In this year Miramichi fry populations averaged about twice as numerous as in any other year between 1951 and 1962. This was true in the Northwest Miramichi and Dungarvon Rivers, which were sprayed in 1954, and in the Cains River, which had never been sprayed. Saunders (1960) found 1955 an outstandingly good year for underyearling salmon in Ellerslie Brook, Prince Edward Island. It was also an excellent year for underyearling trout in the same stream (Saunders and Smith, 1955). In the Margaree River, Nova Scotia, continuing annual assessments of young salmon populations began in 1957. In that year the large parr, over 90% 2-year-olds, were almost half again as abundant as in the next 4 years (indices 13, 9, 8, 8, 9). This too could imply a particularly successful hatch of fry in 1955. In the Pollett River in southern New Brunswick, studies of underyearling salmon survival from potential egg deposition ( = pound of female salmon entering the river in the preceding autumn) were carried on from the 1953 entry of adults to the 1961 hatch of fry. Beginning in 1954, survival rates were 6, 8, 7, 3, 2, 4, 3, and 2%. The highest rate of survival for any year-class was shown by 1955 underyearlings. It thus appears that the unusual abundance of salmon underyearlings in 1955, which happened to be the first post-spray year in the Miramichi area, should be attributed primarily to good conditions for spawning of salmon in 1954 or for survival of fry in 1955, an explanation offered by Keenleyside (1959). He suggested that

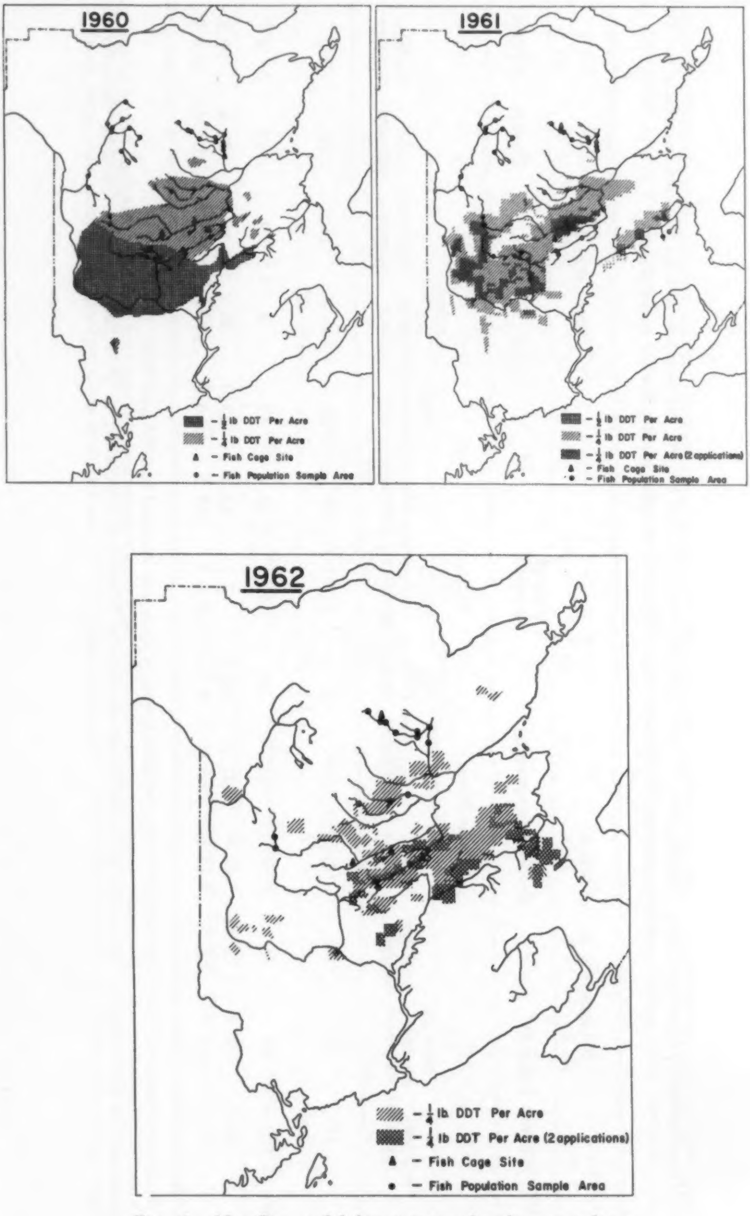
1960
▩ – ½ lb DDT Per Acre
▨ – ¼ lb DDT Per Acre
▲ – Fish Cage Site
● – Fish Population Sample Area
1961
▩ – ½ lb DDT Per Acre
▨ – ¼ lb DDT Per Acre
▩ – ¼ lb DDT Per Acre (2 applications)
▲ – Fish Cage Site
● – Fish Population Sample Area
1962
▨ – ¼ lb DDT Per Acre
▩ – ¼ lb DDT Per Acre (2 applications)
▲ – Fish Cage Site
● – Fish Population Sample Area

FIG. 9. New Brunswick forest sprayed and young salmon assessment stations used in 1960, 1961, and 1962.

TABLE VIII. Recovery of underyearling Atlantic salmon after spraying with ½ lb DDT/acre, expressed as indices ( = mean numbers per 100 yards²) and their standard errors (SE = s/ $\sqrt{N}$) for all samples having similar spray history.

| | No spraying on year-class | | | | | | | After spraying | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | From Table IV | | Before any spraying | | | | Year of spraying | 1 year | | | | 2 years | 3 years |
| | | | | Cains River | | | | | | | | | |
| | All samples[a] | All Miramichi samples[a] | All samples | 1955 only | 1956–59 | Excluding 1955 data | Table IV, col. 2, all samples | All samples | 1955 data only | Excluding 1955 data | Selected samples | All samples | All samples |
| | (1) | (2) | (3) | (4)[a] | (5)[a] | (6) = (3)–(4) | (7)[b] | (8) | (9) | (10) = (8)–(9) | (11)[c] | (12) | (13) |
| No. of samples | 129 | 97 | 58 | 4 | 16 | 54 | 44 | 28 | 19 | 9 | 8 | 15 | 5 |
| Index | 24.2 | 28.4 | 24.3 | 47.9 | 22.2 | **22.6** | 2.7 | 44.5 | 48.2 | 36.9 | **26.0** | **22.6** | **19.1** |
| SE | ±2.1 | ±2.5 | ±2.4 | ±11.8 | ±4.3 | ±2.3 | ±1.4 | ±5.9 | ±6.7 | ±11.8 | ±5.2 | ±5.1 | ±6.8 |

[a] Comparison based on four upper stations only, because lowest station was sprayed in 1956 and 1957.

[b] Table IV indicates several much lower values for areas well within spray boundaries and not receiving hatchery plantings before sampling.

[c] Same samples as in column 10 less one in Little River, tributary of Northwest Miramichi, in 1959 (see text under heading "Recovery in General").

TABLE IX. Recovery of small (10 cm or less from snout to tip of extended tail and commonly 1+years old) Atlantic salmon parr after spraying with ½ lb DDT/acre expressed as indices ( = mean numbers per 100 yards²) and their standard errors (SE = s/ √N) for all samples having similar spray history.

| | No spraying on year-class | | | | | | | After spraying[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | From Table VI | | Before any spraying | | | | Year of spraying | 1 year | 2 years | | | 3 years |
| | | | | Cains River | | | | | | | | |
| | All samples | All Miramichi samples | All samples | 1956 only | 1955 & 1957–59 | Excluding 1956 data | Table VI, col. 2, all samples | Table VI, col. 3, all samples | All samples | In 1956[b] only | Excluding 1956[b] data | All samples |
| | (1) | (2) | (3) | (4)[b] | (5) | (6) = (3)–(4) | (7) | (8) | (9) | (10) | (11) = (9)–(10) | (12) |
| No. of samples | 99 | 78 | 61 | 4 | 17 | 57 | 27 | 24 | 15 | 6 | 9 | 10 |
| Index | 18.0 | 22.5 | 19.5 | 15.8 | 7.9 | 20.7 | 7.3 | 0.6 | 31.1 | 43.9 | 22.5 | 22.7 |
| SE | ±1.6 | ±1.8 | ±1.8 | ±4.4 | ±1.6 | ±1.9 | ±1.5 | ±0.2 | ±4.9 | ±8.7 | ±3.9 | ±6.5 |

[a] After spraying, rates of growth were slower so that these columns may contain, in addition to most 1-year-old fish, a higher proportion of 2-year-olds than occurred under pre-spray conditions.

[b] Small parr of 1956 are separated in this way because they were derived largely from the exceptionally abundant underyearlings of 1955.

TABLE X. Recovery of large (over 10 cm from snout to tip of extended tail and commonly 2+years old) Atlantic salmon parr after spraying with ½ lb DDT/acre, expressed as indices ( = mean numbers per 100 yards$^2$) and their standard errors (SE = $s/\sqrt{N}$) for all samples having similar spray history. (The only data available on 3-year-old parr are from the upper sampling stations of the Northwest Miramichi, where population indices were always comparatively high. Column 7 is therefore given for comparison with column 11.)

| | No spraying on year-class | | | | | | | | After spraying[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Before any spraying | | | | | Year of spraying | 1 year | 2 years | |
| | From Table VII | | | Cains River | | | | | | | |
| | All samples | All Miramichi samples | All samples | 1957[b] only | 1955 1956, 1958, 1959 | Excluding 1957 data | Same stations as col. 11 but before any spraying | Table VII, col. 2, all samples | Table VII, col. 3, all samples | Table VII, col. 4, all samples | Year-classes never sprayed |
| | (1) | (2) | (3) | (4) | (5) | (6) = (3)–(4) | (7) | (8) | (9) | (10) | (11)[b] |
| No. of samples | 80 | 66 | 61 | 4 | 17 | 57 | 17 | 17 | 19 | 18 | 18 |
| Index | 11.5 | 12.0 | 8.0 | 8.5 | 4.5 | 8.0 | 16.0 | 6.3 | 2.8 | 4.8 | 16.2 |
| SE | ±1.2 | ±1.2 | ±1.2 | ±0.2 | ±0.2 | ±1.3 | ±3.5 | ±1.2 | ±0.5 | ±1.1 | ±1.9 |

[a] Immediately after spraying, rates of growth were slower in most places sampled so that these columns may contain more 3+year-old fish than occur in pre-spray columns.

[b] Large parr of 1957, being derived largely from the very abundant class of 1955, are excluded from columns 6 and 11.

lack of competition with parr for food and space, and abundance of food for young-of-the-year, might also have been factors in sprayed streams.

Because of the unusual abundance of the 1955 year-class, Tables VIII, IX, and X are arranged so that conditions associated with it can be removed from consideration.

### RECOVERY IN GENERAL

If data for 1955 are excluded from analysis, the case for higher-than-usual production of underyearlings 1 year after spraying almost disappears (Table VIII). Only one sample, taken in the Little River tributary of the Northwest Miramichi in 1959, showed such increase. But a sample, not sprayed in 1958, taken in the main stem of the Northwest Miramichi 200 yards above the confluence with Little River also showed a substantial increase that same year. This suggests local abundance of spawning stocks in 1958 as a probable explanation. Column 11 of Table VIII gives the mean underyearling index for all stations 1 year after spraying where no other recognized factor operated. The index (26.0 ± 5.2) is only a little greater than that (22.6 ± 2.3) derived from all samples excluding 1955 data. Also, it is not greatly different from indices (24.2 ± 2.1 and 28.4 ± 2.5) derived from all unsprayed samples whether or not there had been spraying 1 or more years before (Table IV).

Whether or not there is a case for minor increase in underyearlings a year after spraying, based largely on 1955 data (Table VIII), the salient feature is that, in the absence of further spraying, recovery to at least the normal condition followed immediately. Recovery, of course, depends on the presence of sufficient parent stock.

Similar information for small and large parr (Tables IX and X) indicates recovery to the normal condition as soon as a new year-class, not subjected to spray, enters the picture.

### CHANGES IN GROWTH AFFECT EARLY RECOVERY

Tables IX and X may include fish of later year-classes in which progress towards smoltification (see "*Smolts*" under heading "Life History and Ecology of Salmon Pertinent to Forest Spraying") was altered by exposure to DDT.

Specimens of young salmon taken after each assessment for numbers were used to study total length (tip of snout to tip of extended tail), weight, and growth patterns as shown by scales.

Some changes in growth patterns of Northwest Miramichi young salmon appear to have followed spraying (Table XI).

Some of the older fish were subjected to spraying in more than one year. For this reason growth increments in particular years were also examined, assuming a direct proportionality between anterior scale radius and total length of fish. Such an assumption lends itself to fairly quick extraction of useful information, and has been applied for salmon (e.g. Jones, 1959) and other fish. Calculation of total length using the direct proportionality assumption introduces error by ignoring the length at scale formation (about 2.5 cm for Atlantic salmon) and an actual curvilinear, rather than linear, scale:body ratio (Kerr, 1961). Calculation of growth increment rather than total length removes much of this error. But growth increment studies on the Northwest Miramichi specimens indicate the same series of growth-to-spray changes as appear in the total length data of Table XI.

TABLE XI. Mean lengths (cm) of 3127 young salmon taken in the Northwest Miramichi River in late August and early September before DDT was applied (1951–53), in years of spraying at ½ lb DDT/acre (upper half in 1954, lower half in 1956 and 1957) and various years after spraying (particular samples, 1955–61). Number of specimens entering each mean in parentheses.

| History | Age-group | | | |
|---|---|---|---|---|
| | 0+ | 1+ | 2+ | 3+ |
| Before spray | 4.7 | 8.3 | 10.8 | 12.9 |
| | (389) | (410) | (193) | (15) |
| Year of spray | 4.2 | 8.2 | 10.9 | 12.8 |
| | (79) | (273) | (291) | (15) |
| 1 year after | 5.2 | 9.6 | 12.4 | 14.7 |
| | (435) | (83) | (113) | (27) |
| 2 years after | 4.1 | 8.2 | 11.4 | 11.6 |
| | (188) | (257) | (24) | (9) |
| 3 years after | 4.9 | 7.9 | 11.0 | 13.2 |
| | (116) | (84) | (108) | (18) |

In spray years young salmon of all age-groups were thin, weighing about 10% less for a given length than before spraying (unpublished data). In the first few years after spraying, body weight for a given length was higher than before spraying except for the largest parr. Further examination is necessary for a clear picture.

Two provisional conclusions seem to be indicated: (1) in the year of spraying, growth of yearling and older parr is curtailed; (2) in the first year after spraying, growth of underyearlings and perhaps of smaller parr is greater than before spraying. But the increased resistance of larger fish to spraying mortality complicates this part of the picture. Early changes in growth rate probably arise from (1) change in the composition of aquatic insect fauna used as food (Ide, 1957, 1961, 1967; Keenleyside, 1967) and (2) altered competitive conditions resulting from mortalities to salmon and other species of fish (Keenleyside, 1959).

Changes in size and age-group distribution produce alterations in composition of smolt runs (Elson, 1957a) which mask the actual reduction in numbers resulting from spraying, as indicated by the following generalizations. (1) Survivors of fish sprayed as *underyearlings* should be a little below normal size at the end of their first year. Above normal growth in the next year should give more 2-year, hence fewer 3-year smolts than normal. (2) Survivors of fish sprayed as *yearlings* should be below normal size as 2-year-olds, many which would otherwise have done so failing to reach the size prerequisite to smolt transformation by the following spring. So the proportion of 2-year smolts from such year-classes would be reduced. Compensatory growth in the third year may result in a greater than normal proportion of 3-year smolts. (3) Survivors of fish sprayed as *2-year-olds* should be below normal size at the end of their third year, reducing the proportion of 3-year smolts, but should nearly all make relatively large 4-year smolts. (4) Survivors of fish sprayed in more than 1 year would be subject to corresponding effects on growth.

Except in sprayed year-classes, total production of presmolt parr has not changed significantly since spraying was begun.

The summation above is based on analyses of samples from several different streams. It is of interest to follow the course of events in two streams, the Northwest Miramichi and the Tobique.

### RECOVERY AFTER A SINGLE SPRAYING

The Northwest Miramichi was first sprayed ($\frac{1}{2}$ lb DDT/acre) in 1954 (Fig. 4). The three upper sampling stations were not sprayed again (Fig. 5, 6, and 9). In Fig. 10 the indices of abundance for the three size-groups of young salmon are shown for the 12 years from 1951 through 1962. Symbols indicate the 1954 forest spraying operation and two other exigencies which affected salmon production. In 1957 extensive forest harvesting operations included piling pulpwood in the river bed near the lowest of the three stations preliminary to "driving" it downstream. Autumn rains failed to materialize before the salmon spawning season and many spawners were barred from the upper river by the piles of logs. In 1960 pollution from a base-metal mine entered the lower part of the river in such concentration and at such time that spawners were prevented from reaching the upper part of the river in normal abundance (Sprague et al., 1965). Figure 10 shows virtual elimination of 1954 underyearlings, but a return to good populations in 1955. This return to normal was maintained throughout the 8 years after spray treatment except as other factors associated with industrial development of the basin limited access for spawners. Unfortunately these other factors occurred at such times as to preclude the possibility of the serious fry kill of 1954 being clearly reflected in a second generation (fry of 1959–61, mostly), if such occurred.

### RECOVERY AFTER SIX SUCCESSIVE ANNUAL SPRAYINGS

The Tobique system was first sprayed in 1953 and some part of it received spray in each of the next 5 years (Fig. 4, 5, and 6). Three times in the 6-year period (1953, 1956, and 1957) most salmon-rearing reaches were sprayed. In the other years, because of spraying on headwater areas the DDT-in-oil could float down on extensive salmon-rearing areas. In 1954 (Fig. 4) only the headwater lakes and streams of one branch were in or bordered on the sprayed area, but this is one of the most important salmon-rearing branches. In 1958 (Fig. 6), the sprayed area covered only about half of the upper drainage basin but included headwater lakes and streams, thus having a disproportionately large effect on salmon-nursery areas below.

Assessment of young salmon populations was carried out, beginning in 1957, at 10 sampling stations for 5 consecutive years. The Department of Fisheries of Canada has maintained records of salmon angling catch here for many

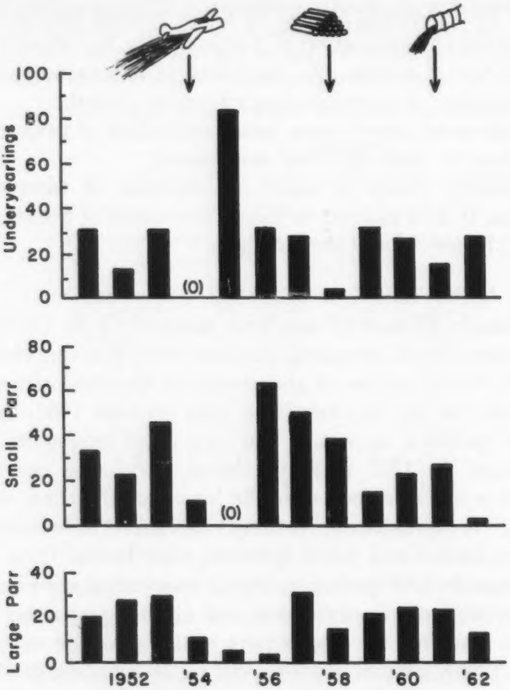

FIG. 10. Recovery of wild young salmon stocks after a single spraying with DDT at ½ lb/acre, shown by changing population indices in the three upper stations of the Northwest Miramichi River. Small parr, over 1 year old but 10 cm and under from tip of snout to tip of extended tail; large parr, over 10 cm (see text under heading "Recovery After a Single Spraying").

years. From 1953 on, the Department counted all salmon entering through a fishway at the confluence with the Saint John River.

Figure 11 presents data arranged vertically to show the relations between parent stock entering one year and their progeny entering 5 years later. The usual year of majority return in the Tobique is that in which 3-year smolts return as grilse and 2-year smolts as 2-sea-year salmon, i.e. the 5th year after entry of parent stocks. These adults were not examined for age and there is minor error in assuming that they were the entire product of parent stocks in the upper row, but years of majority return are well represented. The two middle rows thus indicate the spray history experienced by the returning fish in the bottom row.

As Fig. 11 shows, all the year-classes in which many of the young were subjected to spraying one or more times (young from parents of 1952–57) survived to give adult progeny (1957–62) generally less than half as abundant

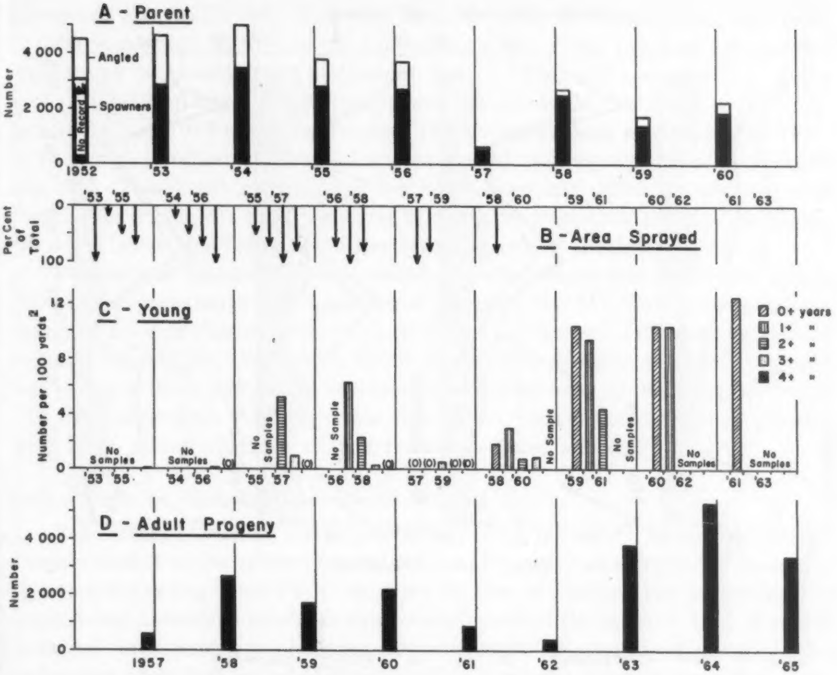

FIG. 11. Recovery of salmon stocks after repeated spraying in the Tobique River. A, numbers of parent salmon entering; B, percentage of rearing areas affected by DDT spray during river life of young derived from A; C, indices of young salmon abundance through the various ages for each year-class, ages being determined from specimens collected during assessment operations; D, numbers of grown salmon in year of majority return for progeny of A (see text under heading "Recovery After Six Successive Annual Sprayings").

as the parent adults. Almost complete spray coverage of the upper basin in 1953 and again in 1957 was followed 4 years later by adult runs under one-quarter of average pre-spray numbers. In 1956, spray coverage of the upper basin, although broad, was less extensive than in 1953 or 1957. Adult salmon returning 4 years later were about half as abundant as earlier runs not affected by spraying.

The head-water spraying of 1958 was followed by the lowest adult run of any year, attributed partly to the location of the spray and partly to the fact that it impinged on the underyearling progeny of the greatly reduced 1957 spawning stock. As young, these 1957 spawners were subjected to one of the heaviest DDT treatments of any New Brunswick salmon — 2 applications of ½ lb DDT/acre over much of the head-water region (Fig. 4). Adults returning in 1957 were also delayed, and some perhaps barred, in their ascent to the Tobique during the construction phase of a large hydroelectric power dam on the Saint John River at Beechwood, 16 miles below the mouth of the Tobique

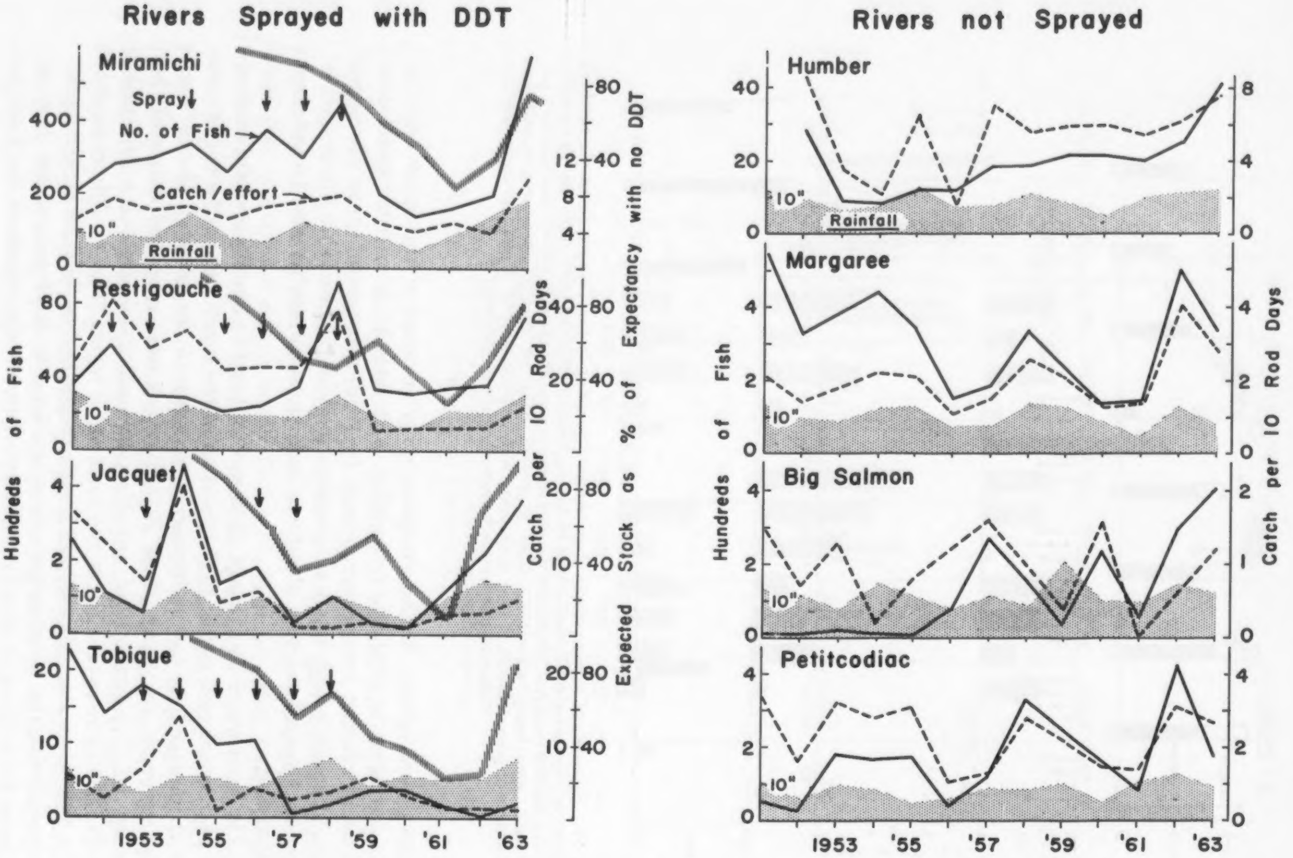

FIG. 12. Atlantic salmon angling catches in DDT-sprayed rivers compared with unsprayed rivers. Upper (hatched) line, predicted effect of spraying on mature stocks as calculated in 1958 for each sprayed stream; solid line, number of fish caught by angling; broken line, catch per 10 rod-days; gray area at bottom of each chart, total June, July, August rainfall from a meteorological station near each river. (Figure from Elson and Kerswill, 1964.)

(Elson and Kerswill, 1964). This may have further reduced spawning that year.

An important feature is the immediate recovery to more or less normal populations produced from the parent run of 1958, with no spraying of the young in 1959 or later. This recovery was maintained by progeny of the comparatively low 1959 run of spawners. The young salmon assessment in 1961, by showing abundant underyearlings, suggested that recovery would continue into 1965. This it did, even though low river water and perhaps some degree of obstruction during the building phase of a new hydroelectric installation nearly 100 miles below the Tobique hindered normal ascent of salmon in 1965.

Recovery in numbers of underyearlings, when there was no further spraying, was commensurate with numbers of parents (Fig. 11). This happened even though there was change in the aquatic insect fauna upon which young salmon depend for food (Ide, 1961, 1967; Macdonald, 1965). Keenleyside (1967) showed that young salmon in sprayed streams altered their food habits to utilize available food organisms. Although this change may contribute to slower growth, there is no direct evidence that it results in lower survival rates.

## Reflection of Spraying in Smolt Runs

Estimation of annual smolt production from the entire Miramichi system comprised part of the salmon investigations. The estimates depended on marking some migrating smolts in freshwater reaches and noting the proportions of marked and unmarked smolts in samples collected in the estuary. C. J. Kerswill (personal communication) believes that although, because of difficulties in the freshwater operation, some of the estimates were of uncertain precision, most of them provide useful measurements of annual smolt production. Analyses of smolt ages in the yearly runs are not complete but before spraying began, the majority of Miramichi smolts were 3 years old (Blair, 1935). For annual runs derived largely from underyearlings hatched before spraying (runs of 1951 to 1956) the average estimate was 1.5 million smolts (range 0.8–2.0 million). The lowest estimate, believed to be fairly reliable, indicated about 0.5 million in 1959, 3 years after the first spraying that covered most of the Miramichi basin (Fig. 5). The highest estimate, over 3 million smolts, was for 1962, 3 years after the only recent year (1959) in which there was no spraying on the system.

Bergeron (1959) counted migrating smolts on the Port Daniel River, Quebec. Proportions of 2- and 3-year smolts varied from year to year, but there were always more 3-year smolts than those of any other age. He found the lowest smolt run to be 3 years after the river was sprayed in 1955.

An association between exposure of underyearlings to DDT forest spray and consequent low smolt runs seems well established.

## Reflection of Spraying in Adult Runs

That extensive DDT treatment of forests can be followed not only by reduced populations of young but also by reduced catches of grown salmon after an appropriate span of years has been discussed by Kerswill (1958) and by

Elson and Kerswill (1964) (see Fig. 12). Fortunately, as already explained, the drastic reduction of young in any one year is spread over several years of returning adults. If underyearling stock were completely wiped out in one year, there would still be a small recruitment of adults even in the year of majority return for the damaged year-class. Such built-in resilience may be overcome, however, when spraying occurs in the same areas in successive years. Similarly, if extensive spraying should impinge on successive generations, as happened on the Tobique in 1953 and 1958, resulting adult stocks would be severely reduced, as they were there in 1962 (Fig. 11). In 1962, spawning stocks there were so low that their progeny returning in 1967 may still reflect depression from sprayings 9 years and more earlier. However, if the progeny of such reduced year-classes are not sprayed, the buffering action of other year-classes should permit a buildup to normal abundance within one or two generations (Elson, 1962c) unless new hazards intervene.

### Transferred Effects of Spraying

Rudd (1964) lists a number of instances in which adverse effects of persistent insecticides were transferred from the originally exposed animals to others. Burdick et al. (1964) recount how all fry from female lake trout previously exposed to DDT died when the eggs were cultured in hatcheries. They concluded that such deaths occurred when the young fry contained more than 2.9 ppm DDT. The parent fish lived in an environment where contact with, and hence accumulation of, DDT was possible over a period of years. But seagoing Atlantic salmon are in contact with the insecticide mainly while they are small. In contrast to the condition described for Sebago Lake salmon (Anderson and Everhart, 1966) any sublethal content contained in their bodies must be greatly diluted by the time of return from the sea, when they have increased their body weight by 40 times (for 3-lb grilse), 120 times (for 10-lb salmon), or more. Mr Roger P. Dexter, Biologist at the Craigbrook Fish Hatchery, Maine, (personal communication) informed me that an analysis of Miramichi young salmon (eggs eyed and hatched in Maine) indicated a DDT-and-derivative content at about one-tenth the level given by Burdick et al. as critical. Records (unpublished data) from the Northwest Miramichi River, where entering adult salmon have been tallied through a weir and quantitative assessments of young made each year since 1951, show no decrease in survival rate between potential egg deposition and late summer underyearlings that can be related to DDT spraying. Nor do records of Canadian government hatcheries, where several million Miramichi salmon eggs are incubated each year, show any decrease in viability comparable to that reported by Burdick et al.

Ferguson et al. (1964) report the development of resistance to chlorinated hydrocarbon insecticides in some species of freshwater fish. There is as yet no information to suggest that the progeny of New Brunswick salmon which survive DDT spraying as young are any more resistant to the pesticide than their parents. But past spray programs have not lent themselves to any definitive examination of this matter.

## ACKNOWLEDGMENTS

It gives me pleasure to acknowledge assistance from others in gathering, analysing, and reporting the material presented here. Dr C. J. Kerswill had charge of Atlantic salmon investigations originating from the Fisheries Research Board of Canada's Biological Station at St. Andrews, N.B., during the period covered. Collecting data on populations of wild fish, sometimes in isolated regions and often under uncomfortable conditions, was handled by crews of field technicians under the several leaderships of Messrs P. R. Graves, E. J. Schofield, and H. P. Barchard. Messrs J. E. Paloheimo and E. L. Cadima gave advice and assistance in the mathematical aspects of planning and analysis. Cooperation of personnel of the Department of Fisheries of Canada, the Canada Department of Forestry, Forest Entomology and Pathology Laboratory, Fredericton, N.B., and Forest Protection Limited, especially Mr B. W. Flieger, facilitated planning of the work and analysis of results. I particularly wish to thank my colleagues on the scientific staff of this Station, especially Dr M. W. Smith, in charge of this Station's Anadromous Fish Investigations from 1963 to 1965, for their helpful criticism. I want, also, to thank Messrs Wm. McMullon and F. Cunningham for preparing the illustrations accompanying this account.

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# Effects of Forest Spraying with DDT on Aquatic Insects of Salmon Streams in New Brunswick[1,2]

By F. P. Ide

*Department of Zoology,
University of Toronto, Toronto, Ontario*

## ABSTRACT

Sampling of emerging aquatic insects by cage-traps on a 24-hr basis showed the effects on the stream fauna of spraying forests with DDT at ½ lb/acre in June. Except for a few individuals that presumably emerged from unaffected pupae and nymphs, there was an interval of several weeks after spraying when no emergence occurred. From about the middle of August, however, large populations of very small insects, mainly chironomids, emerged in the spray year. The year after spraying, as compared with the spray year, there was an increased variety including some larger species.

In following years there was further recovery by increase in larger forms through reproduction from residual small populations. The caddisflies recovered more slowly than other groups. After a single application of spray the fauna had practically recovered qualitatively in 2 or 3 years in some groups but usually not for 4 years or more in caddisflies. With a further similar spray applied within 3 years a reduction occurred equal in severity to that of the original spraying.

From the standpoint of feeding of the young salmon, significant facts are: (*a*) the severe reduction in the bottom fauna of larvae and nymphs of all sizes in the first weeks after spraying; (*b*) the large populations of small larvae, mainly chironomids, developing in late summer of the spray year and so available to fry; (*c*) increasing numbers of larger insects the year after spraying and subsequent years.

## INTRODUCTION

AERIAL SPRAYING with DDT of large tracts of forest in New Brunswick was carried out from 1952 to 1962 with the exception of 1959. A Crown company, Forest Protection Limited, carried out the spraying program, the purpose of which was to control an outbreak in the balsam and spruce forest of the spruce budworm, *Choristoneura fumiferana* (Clem.). This insect has periodically increased to serious infestation numbers in eastern Canada causing much destruction to the forest trees. Details concerning the southward movement of the budworm and the spray operations are outlined by Webb et al. (1961). The details of spray application from planes and other relevant data are outlined by Kerswill (1967).

The Miramichi River is one of the most important salmon rivers in eastern Canada. In 1950 it was selected by the Fisheries Research Board of Canada as

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[2] The fourth in a series of papers on work of the Fisheries Research Board of Canada on effects of forest insecticides on salmon and aquatic insects.

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the site of long-term investigation of the causes of variation in salmon populations. Kerswill (1967) explains how forest spraying with DDT became important in the salmon's environment, leading to inclusion of the new factor in the investigations.

As part of this program an investigation was begun on the effects of the spraying on food organisms in the streams, particularly the aquatic insects, an important component of the diet of young salmon. A cursory survey of sprayed and unsprayed streams of the Northwest Miramichi system was made approximately a month after spraying in 1954. Major differences were noted between some sections of the sprayed streams and unsprayed tributaries of comparable character. At two sprayed locations, one at Station S (Fig. 1) on the North Branch of the Sevogle River, a tributary of the Northwest Miramichi, the other at Crawford Pool (near N) on the Northwest Miramichi itself, no insects were apparent in an examination of several stones picked from the bottom, although other organisms were observed, including aquatic mites, snails, and planarians. A similar absence of insects was noticed farther down the Northwest Miramichi at Camp 42 (Station N). The stones of the bottom at these three places were densely coated with algae, making them slippery and altering the appearance of the bottom. At a fourth location, a small stream in the sprayed area, the only insects observed were numerous small chironomid larvae which had recently hatched. By contrast, two unsprayed streams of the same drainage system, Trout Brook (Station T) and Millstream Brook (Station M), contained insect nymphs and larvae in the variety and abundance characteristic of the region. This examination of the stream bottom a month after spraying gave, however, an erroneous impression of the severity of the effect of spraying. Subsequent examinations indicated that many insects survived, some in the egg stage, some as pupae, and a few of the more tolerant species as nymphs or larvae.

Intensive sampling of the aquatic insects in some sprayed and unsprayed streams of the Northwest Miramichi River system was begun by the Biological Station of the Fisheries Research Board of Canada at St. Andrews in 1955 and continued each summer to 1962 under the author's supervision. A preliminary report (Ide, 1957) has been made for 1955 and 1956 in which methods have been given in detail.

## RIVER SAMPLING STATIONS

In Fig. 1 the Northwest Miramichi River is shown, with the locations of the insect sampling stations and the spray areas. Information on the river system is given by Kerswill (1967).

Station S on the North Branch of the Sevogle was operated 1955-56-57-58 and 1962 and was in the spray areas of 1954, 1957, and 1958. This location was selected because of the severe effect of the 1954 spray on the aquatic insect fauna observed in the cursory examination of the bottom. Since the area was resprayed in 1957 and 1958 there was no opportunity of following the recovery in insect populations affected by the 1954 spraying. It therefore was discontinued

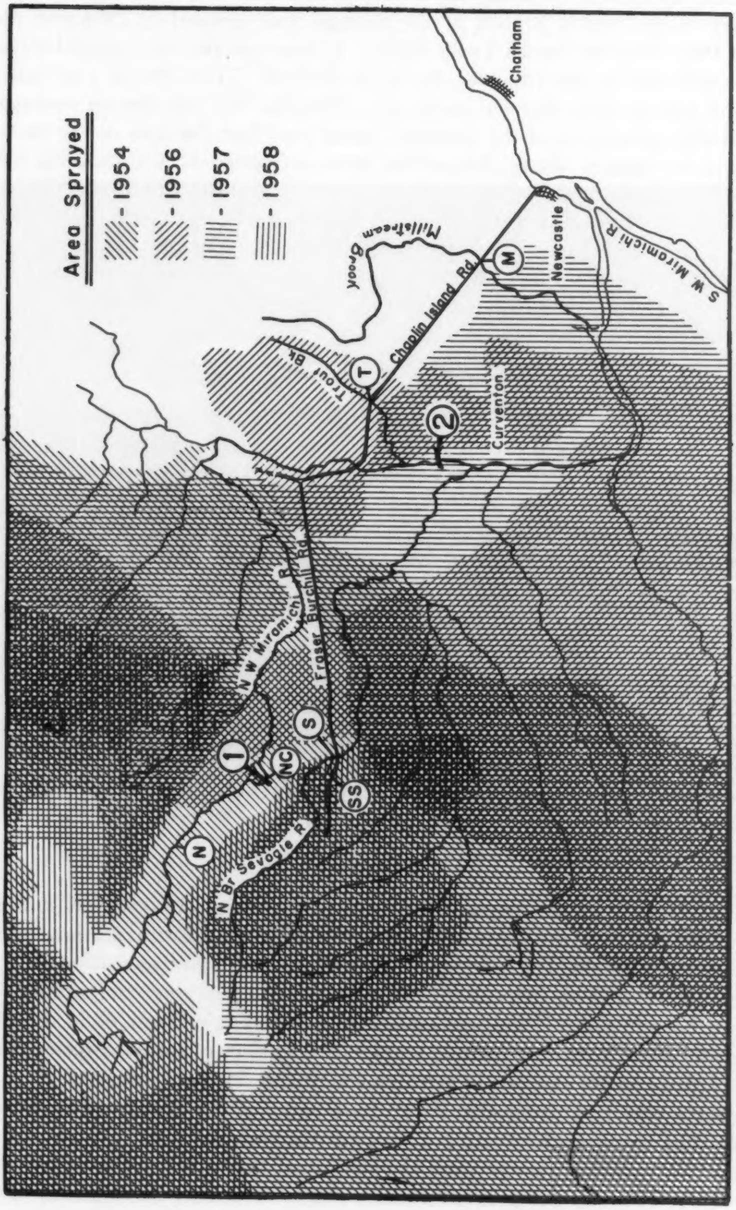
Area Sprayed
▨ - 1954
▨ - 1956
▨ - 1957
▨ - 1958
Chatham
Millstream Brook
Newcastle
S W Miramichi R
M
Chaplin Island Rd
T
Trout Bk
2
Curventon
N W Miramichi R
Fraser Burchill Rd
1
NC
S
SS
N
N Br Sevogie R

FIG. 1. Northwest Miramichi River showing locations of sampling stations and spray areas.

after 1958 except for checking in 1962. An adjacent station, SS, on a small tributary of the North Branch of the Sevogle was sprayed in 1954 and used only in 1955. The station on Trout Brook, T, was selected as a control stream in 1955 and was in operation for the years 1955–62. Trout Brook was outside the spray area of 1954, but was sprayed in 1956. In 1957 this stream was again sprayed downstream from the Chaplin Island road but the area of the station itself and the stream above the station were not sprayed. A station on Millstream Brook, M, was then selected as a control, particularly to assess effects of spraying on the Sevogle, because of its remoteness from the 1954 spray area. It was maintained from 1955 until 1958 and then discontinued. Although not sprayed in 1957 it was very close to the upper border of the spray area and may have been affected somewhat. The stream differed from the North Branch of the Sevogle in the more discontinuous nature of its rapids at the station site and in the fact that it emptied into the estuary of the Northwest Miramichi below all rapid sections.

The Camp 42 station, N, on the Northwest Miramichi was established in 1957 and was maintained through 1961. It was selected as a substitute for the station on the North Branch of the Sevogle since it had the same spray history originally and, in the subsequent cursory examination of the bottom in 1954, showed a similar extreme reduction in insects. Also, the bottom presented much the same condition with respect to its fauna as the North Branch of the Sevogle. Further, Camp 42 was in a reserve area exempted from spraying after the initial spraying of 1954. Camp Adams, NC, about 10 miles downstream from Camp 42 on the Northwest, was selected in 1959 to supplement the station at Camp 42 and was operated until the fall of 1962. It was less affected by the 1954 spray than Camp 42, judging by the stomach contents of fish taken in the area. However, an area of forest bordering the north side of the river from a point approximately 3.5 miles upstream from Camp Adams and extending for about the same distance downstream was sprayed in 1956 (Fig. 1) and this may have affected the station.

## METHODS

The changes in the populations of aquatic insects of sprayed and unsprayed streams over the years 1955–62 were followed at several stations on the Northwest Miramichi. The sections of the streams designated as stations (Fig. 1, 3) were determined by inspection. All were in rapids having comparable flow rate and bottom type. They were selected in different streams or in different parts of the same stream to include areas with a variety of spray histories. They also included control stations in streams which had not been sprayed.

Insects emerging from the water were sampled by means of cage-traps (Fig. 2). The traps, each covering a yard-square area (0.915 $m^2$), catch and retain the insects which emerge from the surface of the water of the rapids (Ide, 1940, 1957). They do not prevent aquatic stages of insects moving into or out of the yard-square area of bottom over which the cage sits but capture the adults emerging from the surface of the enclosed area. To ensure that the traps

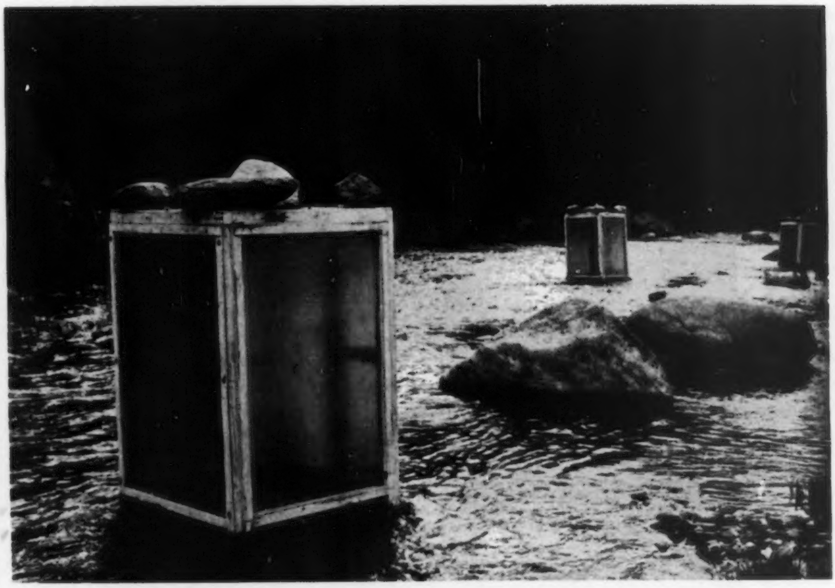

FIG. 2. Close-up of cage-traps in position at North Branch of Sevogle River (Station S).

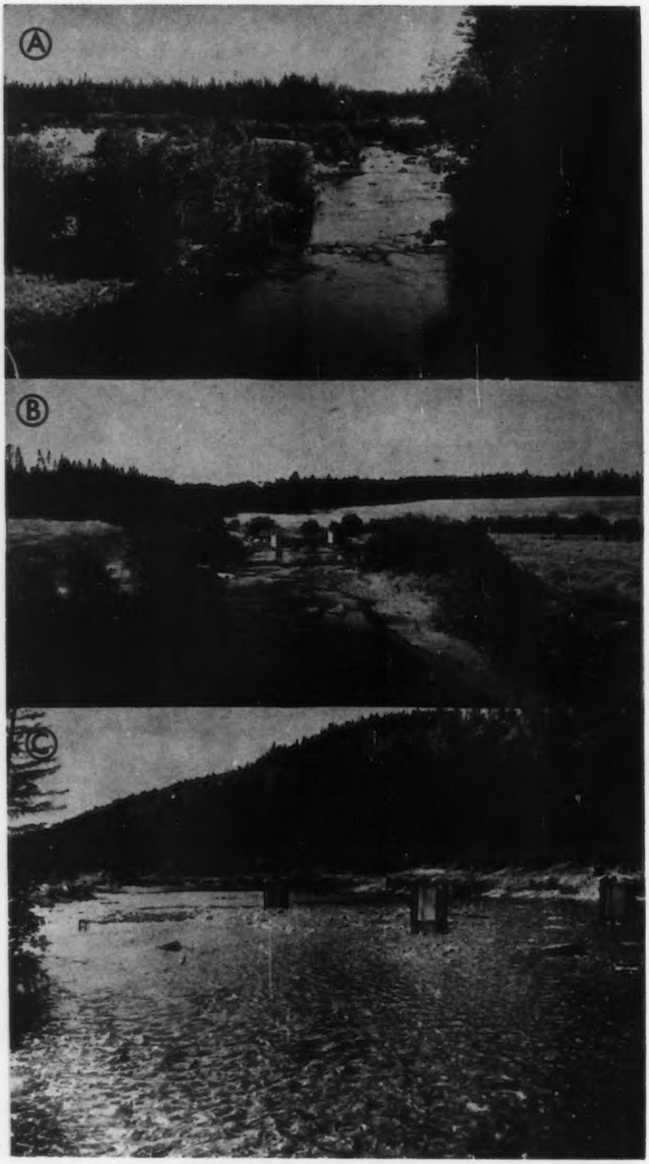

FIG. 3. A, Trout Brook, below Station T; B, Millstream Brook, unsprayed control (Station M); C, Northwest Miramichi at Camp 42 (Station N).

would retain the smaller insects, three sides and the top were covered with a glazed wire screening, "Windowlite," and the fourth side with 24-mesh (10 per cm) copper screening (0.707-mm apertures), rather than being entirely of 15-mesh copper screening as specified in the original description (Ide, 1940).

In 1955 a pair of yard-square cage-traps were employed at each station, but in all later years three such traps were used at each station. These were designated by numbers, for example T1, T2, and T3 for Trout Brook, T1 being in the upstream position. Collecting was begun in late May or early June and continued until late August or early September, the period over which most emergence takes place. The cage-traps were cleared of insects at approximately the same hour of the day so that collections gave the number retained over a 24-hr period. At Camp Adams, in 1962 only, the procedure was modified when for a 72-hr period in the last week of August collections were made at hourly intervals. This presumably gave somewhat higher readings (Sprules, 1947), but would result in only a negligible percentage difference in the annual figures for 1962 as shown in Fig. 11 and 12 and Appendix VI. In 1955, six collections were made from each cage each week. In 1956 and later years, five collections were made from each cage. Since the total number of collections varied in the different summers, the results for annual totals were made comparable by changing them all to 60-day equivalent values. The figures used in comparing emergence in different years are, therefore, calculated values for insects emerging during 60 24-hr periods in a period of about 100 days of the summer. Summary data are provided in Appendices I–VI.

The insects were collected from the inside surfaces of the cage-traps using a moistened swab, or with forceps for the larger individuals, and placed in vials containing 70% alcohol. They were identified under a binocular microscope, counted, and volumes determined by displacement of water in a 5-ml graduated cylinder.

Results of collections from the cage-traps are presented in the following three ways.

(*1*) As histograms showing daily emergence of various groups of insects (Fig. 4–6).

(*2*) As graphs showing the emergence of all insects and of chironomids from week to week (Fig. 7–9).

(*3*) As graphs and tables showing the emergence of insects of major groups from year to year (Fig. 10–12, Appendices I–VI). The summer refers to the period from approximately June 1 to September 7. In these graphs, a solid line is drawn for the average of two cage-traps in 1955 and three in other years, and the original points are also shown. High and low numbers are connected with dotted lines to show spread in numbers between cage-traps. The spread in these points is great in some cases but with few exceptions the trends are similar. The objective in selecting cage sites was to place the cages over similar bottom with similar depth and rate of flow. The differences in the samples probably reflect mainly differences in bottom, which were not apparent at the time of making the selection, and seasonal changes in current, which were difficult to anticipate. The number of organisms taken in a cage was generally low where the stones were partially imbedded in silt or sand, but high where moss grew on the stones; these features were more pronounced for some groups of insects than for others.

## DAILY EMERGENCE OF INSECTS OF MAJOR GROUPS (EXCEPTING CHIRONOMIDS)

### TROUT BROOK (STATION T) (FIG. 4)

Daily emergence of insects of major groups, excepting chironomids, is shown in Fig. 4 for Trout Brook for 1955–62. The spray was applied June 14–17, 1956, as indicated by an arrow. An extremely heavy freshet occurred in late May in 1961 before sampling began.

*Mayflies* (Ephemeroptera) emerging in 1956 were greatly reduced in numbers after the spray (Fig. 4A). In 1957 there was an increase, most marked in the second half of the summer and accounted for mainly by second generation individuals of small species. By 1958 the early emerging individuals were again well represented. These were mostly early univoltine species (one generation per year), or first generations of bivoltine species (two generations per year), which had been affected by the spraying in mid-June 1956. The numbers emerging were highest in 1959, higher than in the pre-spray year, and declined through 1960 and 1961. In 1961, the numbers emerging early in the season were particularly low. The extremely high flooding in May of 1961 may have been a factor contributing to this, for in 1962 there was a marked increase in numbers as compared with 1961. These insects are mainly herbivores.

*Stoneflies* (Plecoptera) were not as numerous as the mayflies, but some of the species were of large size. The numbers were higher in 1956, the spray year, than in 1957, when they were lowest (Fig. 4C). But the 1956 numbers include those for the 2 weeks before spraying when early species of stoneflies were emerging. The numbers were high in 1958, somewhat lower in 1959, high in 1960, reduced below the pre-spray year again in 1961, and up in 1962. This group includes some predatory species.

*Caddisflies* (Trichoptera) emerged in small numbers before the spraying in June 1956, but after the spraying a large number emerged in July (Fig. 4D). Nearly all these were species of case-bearing Rhyacophilidae of the subfamily Glossosomatinae which were in the pupal stage at spray time and apparently little affected. Some of the individuals emerging in late summer were of two species of limnephilids which diapause in the larval stage during the summer months and were apparently unaffected by the spray. In 1957 the numbers of caddisflies were minimal and much below the numbers of the pre-spray and spray years. The numbers were highest in 1959. The two peaks in both 1959 and 1960 show the re-establishment of the early and late summer generations. In 1961 the numbers were low again, much below the pre-spray numbers. The almost complete lack of emergence in June 1961, may reflect the effect of extremely high floods in late May of that year. The 1962 numbers were appreciably higher than those of 1961.

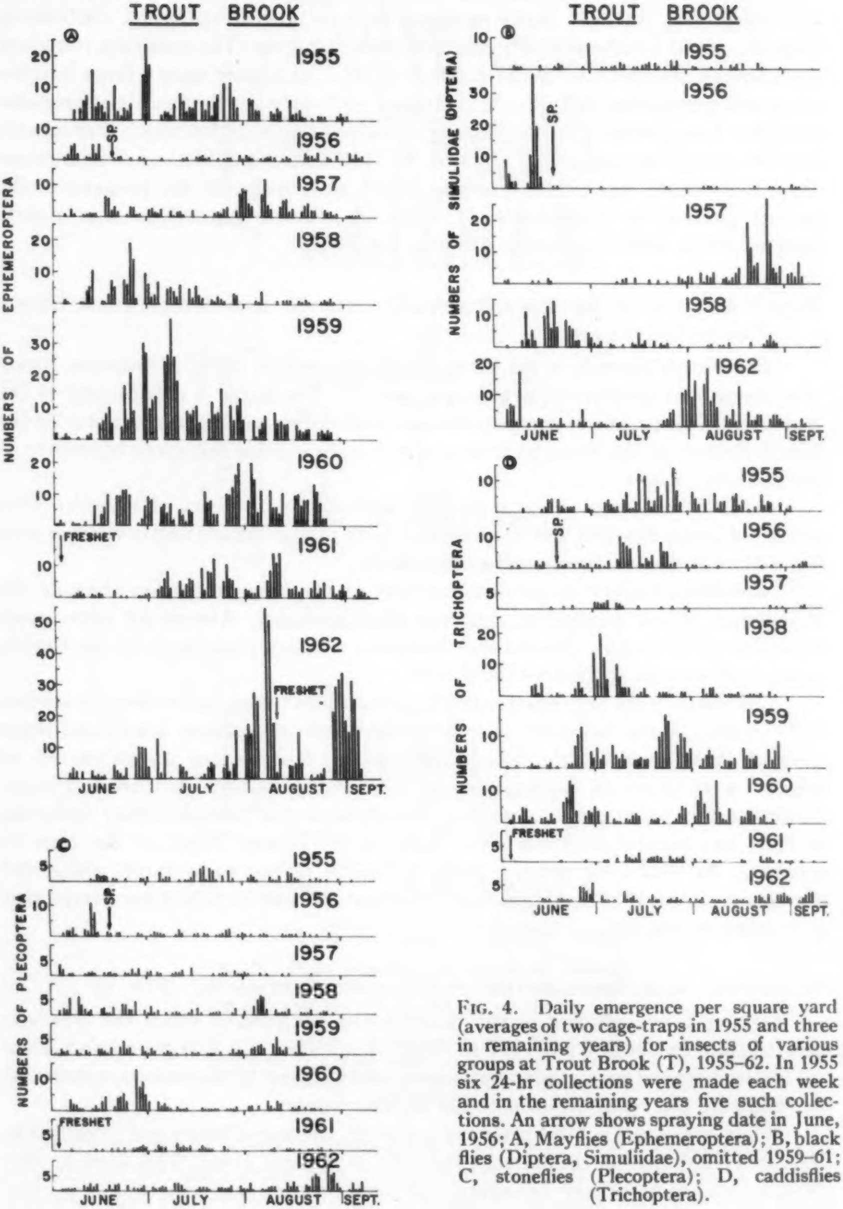

FIG. 4. Daily emergence per square yard (averages of two cage-traps in 1955 and three in remaining years) for insects of various groups at Trout Brook (T), 1955–62. In 1955 six 24-hr collections were made each week and in the remaining years five such collections. An arrow shows spraying date in June, 1956; A, Mayflies (Ephemeroptera); B, black flies (Diptera, Simuliidae), omitted 1959–61; C, stoneflies (Plecoptera); D, caddisflies (Trichoptera).

*Black fly* (Diptera: Simuliidae) emergence was low in 1955, the pre-spray year (Fig. 4B). In 1956 many emerged in June, before spraying, contrasting with the small numbers which emerged after spraying. The numbers remained low through the first half of the summer of 1957, and there were a large number of second generation individuals in August and early September. It is probable that this large group was the progeny of ovipositing females which immigrated into the area from unsprayed streams. In 1958 a large population of first generation individuals was evident, among which were probably the progeny of the second generation individuals of 1957. A second generation was poorly represented in 1958, but well represented in 1962.

### NORTH BRANCH OF SEVOGLE RIVER (STATION S) AND MILLSTREAM BROOK (CONTROL-STATION M) (FIG. 5)

The North Branch of the Sevogle was sprayed in 1954; Millstream Brook was unsprayed and was selected as a control. The latter is a tributary of the Northwest Miramichi of comparable size and rather similar in character to the North Branch of the Sevogle; it was over 5 miles outside the spray boundary at the sampling point.

In 1955, the year after spraying, fairly large numbers of *mayflies* were produced in the Sevogle, yet the numbers both of individuals and of species were fewer than in the Millstream Brook control.

*Stoneflies* emerged in greater numbers at the Sevogle station than at the Millstream Brook control in the year after spraying. Almost all were small stoneflies of the genera *Leuctra* and *Nemoura*, the latter and some of the former being early-season species.

*Caddisflies* were not represented in the samples taken at the Sevogle station in 1955, and in the following year only eight individuals were taken and these were of three species only. Many individuals, representing an estimated 16 species, were taken in the samples in 1955 at the Millstream Brook control. Unfortunately no intensive sampling was done of the Sevogle before spraying in 1954 but some collections were made of the insects killed at the time of spraying. At that time several kinds of caddis larvae were found which did not appear in the 1955 sample series, an indication that there had been reduction in number of species.

### NORTHWEST MIRAMICHI RIVER AT CAMP 42 (STATION N) (FIG. 6)

Station N was studied briefly in 1954, the one year in which the area was sprayed, and in detail from 1957 to 1961. It replaced the Sevogle station when the latter, because spraying was repeated in 1957 and 1958, became unsuitable for following the rehabilitation of the aquatic insects.

When examined in 1954 after the spraying, the insect fauna was found to be drastically reduced as compared with that in control areas. The river at this point seemed to have been as severely affected by the spray as it was at a point 2 miles upstream and as the Sevogle was at station S. It is impossible to know

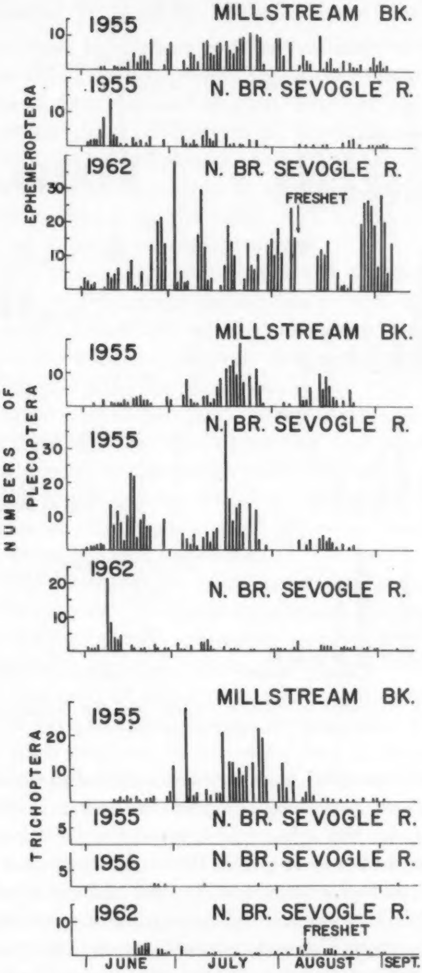

FIG. 5. Daily emergence of insects of various groups at Millstream Brook (M) (unsprayed control) and the North Branch of the Sevogle River (S) (sprayed in 1954, 1957, and 1958). The comparison is of results for Millstream Brook in 1955 with those for the North Branch of the Sevogle in 1955 (1956 also for Trichoptera) and 1962.

definitely the trends in insect populations in 1955 and 1956, although these can be surmised by comparison with the North Branch of the Sevogle, a stream of similar type and similarly affected by spray in the same year, and with Trout Brook, which was sprayed in 1956.

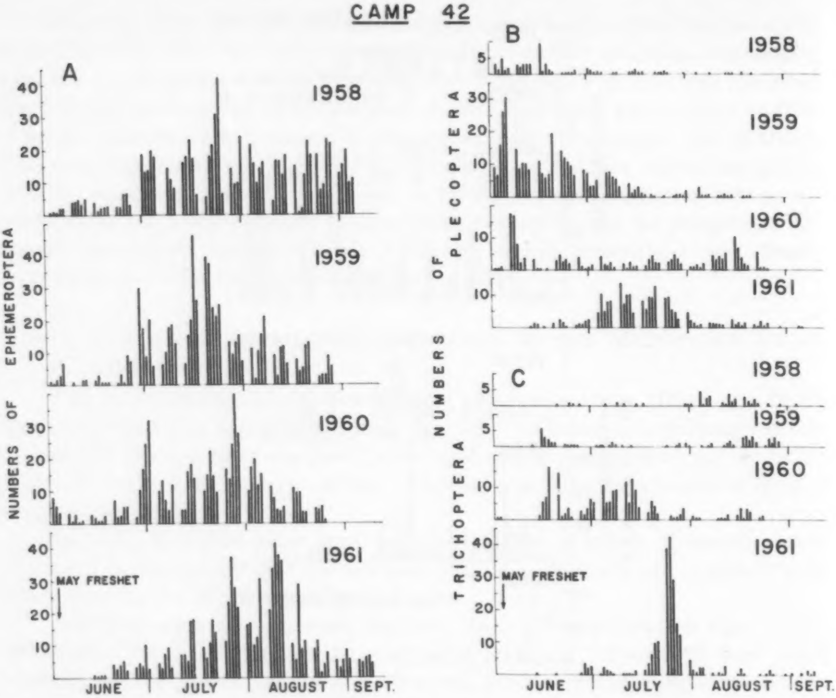

FIG. 6. Daily emergence of insects of various groups from the Northwest Miramichi River at Camp 42 (N) (sprayed in 1954 only).

From 1958 to 1961 *mayflies* were well represented in numbers of individuals (Fig. 6A). The numbers had been considerably lower in 1957, however (Appendix V). *Stoneflies*, on the other hand, were low in numbers of individuals in 1958 (in 1957 also) but were high in 1959 and decreased somewhat in 1960 and 1961 (Fig. 6B). *Caddisflies* were low in 1959 and the 2 preceding years, and it was not until 1960 that a substantial increase occurred (Fig. 6C). This was a year later than for the stoneflies and 2 years later than for the mayflies. The lag in recovery of this group of insects at this station was 5 years, in comparison with 3 years for Trout Brook. The recovery in populations of caddisflies was mainly accounted for by many individuals of a rather small number of species as compared with Millstream Brook (control).

## WEEKLY EMERGENCE OF ALL INSECTS AND OF CHIRONOMIDS

Figures 7–9 show the emergence of all insects and of chironomids from week to week, as average numbers emerging per square yard per day, and, similarly the average volumes of all insects. The chironomids (Diptera) are small and very numerous insects, so the curves of their numbers generally parallel closely those of the numbers of all insects.

### TROUT BROOK (STATION T) (FIG. 7)

In the pre-spray year 1955 there were rather large numbers of the larger insects (that is, those other than chironomids), a correspondingly high volume, and two main peaks of emergence. The early summer peak consisted mainly of univoltine species and first generations of bivoltine species and the later summer peak mainly of the second generations of bivoltine species. In the spray year 1956 there was an immediate drop in emergence after spraying to a low figure that coincided in time with the early peak in 1955. This illustrates the initial drastic effect of spraying on emergence.

Although the drop in numbers emerging in the 3 weeks after spraying was pronounced, some insects did emerge during this period, including rather large numbers of a case-making caddisfly of the subfamily Glossosomatinae; these are presumed to have been in the relatively non-vulnerable pupal stage at the time of spraying. Figure 7 also shows strikingly the 1956 emergence of many chironomids in the latter half of July and the first half of August. Most of them probably developed from eggs laid before spraying, and the remainder from eggs laid by insects which were in the pupal stage during spraying and survived. The large numbers of chironomids coincided with a scarcity of other insects which ordinarily compete with them for food (e.g. some mayflies), or which prey on them (e.g. some stoneflies and caddisflies).

In 1957 and 1958 chironomids were again predominant, peak numbers being earlier in the latter year. The large numbers of late July and early August in 1956 were replaced by an almost coincident trough by 1958. In 1959 numbers of chironomids were high, especially from mid-June to the end of August. In 1960 they were low, not only in absolute numbers but also relative to the numbers of other aquatic insects. The pattern of emergence was notably different than in 1959, with high numbers in the latter half of June and from the first half of August to September. The emergence in 1960, the fourth after spraying, was thus more similar to that of 1955, the pre-spray year, than to any earlier post-spray year. In 1961 the numbers of insects emerging were lower than in any other year of sampling, the small numbers of chironomids being the most significant factor. Insects other than chironomids were relatively more numerous and the volume disproportionately higher than when smaller insects predominated. Many of the larger insects, such as some of the stoneflies and caddisflies, are known to feed on chironomids and other small insects in the water (Muttkowski and Smith, 1929) so that changing numbers of chironomids relative to those of other insects may reflect utilization of the former as food. In 1962, however, the numbers of chironomids and all other insects were higher than in 1961 with an accompanying increase in bulk. Extreme flooding of near record height in May 1961, causing removal of silt and drastic modification of the stream bottoms, was probably a factor in the low populations of 1961.

### NORTH BRANCH OF SEVOGLE RIVER (STATION S) (FIG. 8)

Station S was sprayed in June of 1957 and 1958; it had also been sprayed

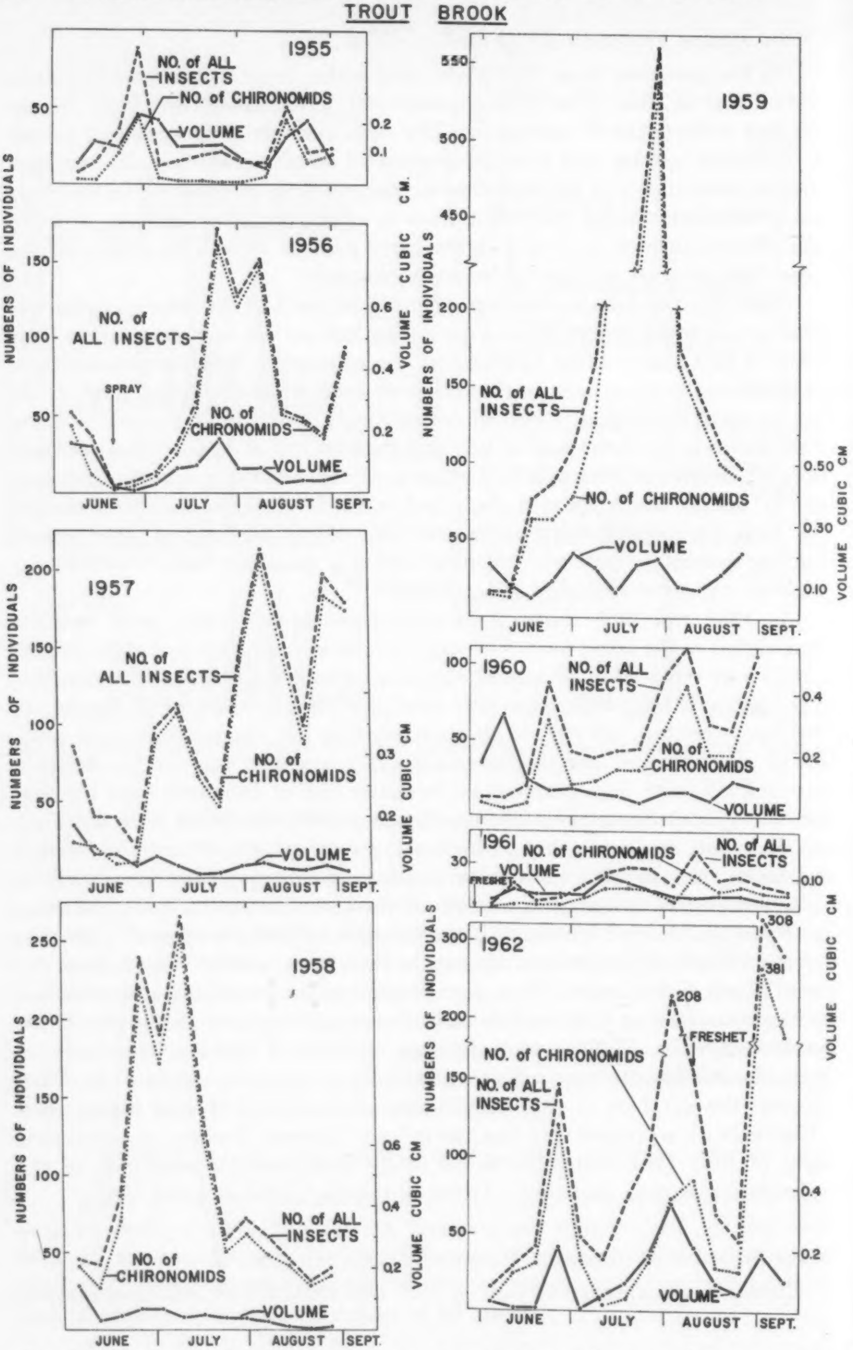

FIG. 7. Emergence of insects from week to week, as average numbers emerging per square yard per day, for Trout Brook (T) (sprayed in 1956 only) for the summers of 1955–62. Numbers of chironomids, numbers of all insects including chironomids, and corresponding volumes are shown. Each weekly point on the graph is the average of 15 yard-square collections (three cage-traps collected for five 24-hr periods) except for 1955, when each point is the average of 12 yard-square collections (two cage-traps for six 24-hr periods).

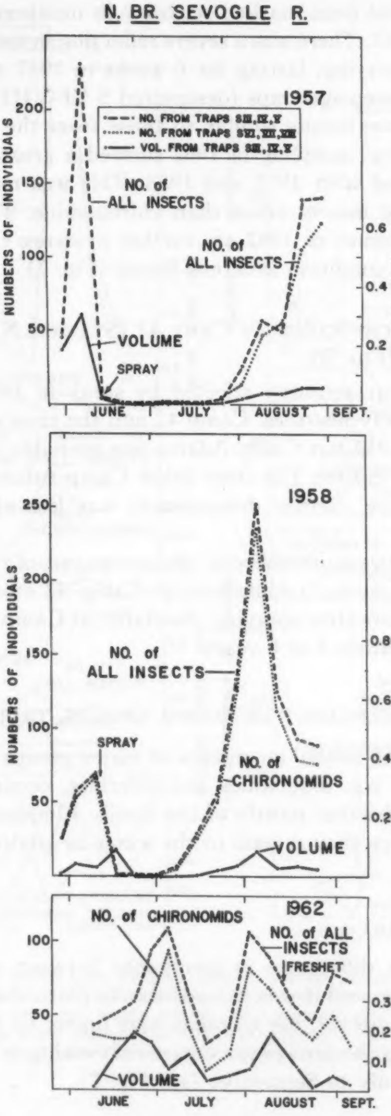

FIG. 8. Emergence of insects from week to week, as average numbers emerging per square yard per day, for the North Branch of the Sevogle River (S) (sprayed in 1954, 1957, and 1958) for the summers of 1957, 1958, and 1962.

in 1954, but there had been marked recovery in numbers of insects emerging between 1954 and 1957. There was a severe reduction in emergence in the period immediately after spraying, lasting for 6 weeks in 1957 and 4 weeks in 1958. A duplicate set of three cage-traps (designated S VI–VIII) placed about 300 ft upstream in 1957 gave results similar to those from the original set. After 4 years without spraying, sampling in 1962 showed a greater volume of insects emerging as compared with 1957 and 1958. This was mainly because of the increased numbers of insects other than chironomids. The prominent peaks in early and late summer of 1962 are further evidence of recovery; they resemble the pre-spray condition at Trout Brook (Fig. 7).

### NORTHWEST MIRAMICHI RIVER AT CAMP 42 (STATION N) AND CAMP ADAMS (STATION NC) (FIG. 9)

Both stations were seriously affected by spray in 1954 but subsequently they had different spray histories. Camp 42 and the river above it was exempt from spraying after 1954 but Camp Adams was probably affected by spraying in 1956 as mentioned earlier. The river below Camp Adams was sprayed again in 1957. Camp Adams, farther downstream, was less affected by the 1954 spray than Camp 42.

If the spread between numbers of all insects and of chironomids is taken as an indicator of recovery, populations at Camp 42 evidently recovered, in general, by 1959, 5 years after spraying. Similarly, at Camp Adams, populations evidently recovered within 5 or 6 years.

## ANNUAL EMERGENCE OF MAJOR GROUPS, TROUT BROOK

Figure 10 indicates annual emergence of major groups of insects for Trout Brook. Other insects, e.g. dragonflies and alderflies, occurred in insignificant numbers. Small riffle beetles, mostly of the family Elmidae, were abundant at some stations but, since they remain in the water as adults, were not taken in collections.

### CHIRONOMIDS (FIG. 10A)

There were great differences in emergence between cages. Trout Brook is a rather small stream and it was not possible to place the three cage-traps in uniform conditions. However, the numbers were higher on the average in 1956, the spray year, than in the pre-spray year, mainly owing to the high emergence of chironomids from July to September (see Fig. 7).

### STONEFLIES (FIG. 10B)

These insects were comparatively low in numbers. They are of moderate to large size, and some species prey on other insects. They were not reduced as greatly in numbers as some of the other groups. The numbers reached a low in the post-spray year, 1957, partly owing to the considerable number which

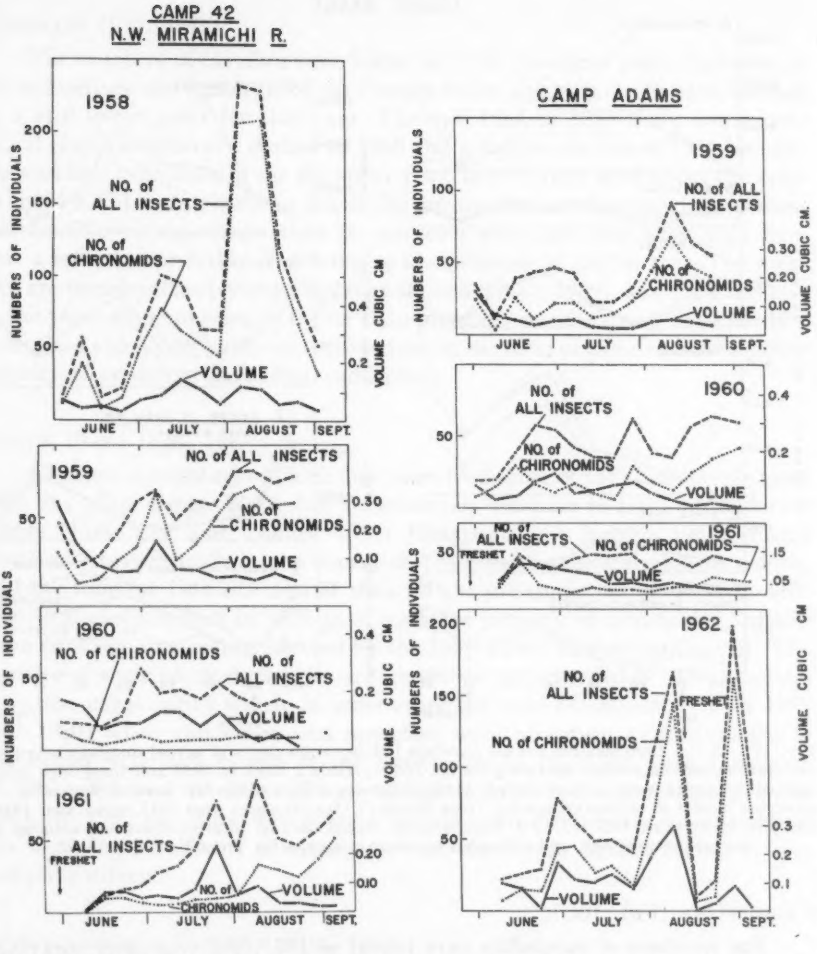

FIG. 9. Emergence of insects from week to week, as average numbers emerging per square yard per day, for two stations on the Northwest Miramichi River: Camp 42 (N) (sprayed in 1954 only) for summers of 1958 to 1961, and Camp Adams (NC) (sprayed in 1954 and probably affected by spray in 1956, when the north bank of the river was sprayed in the vicinity) for the summers of 1959 to 1962. Camp Adams was near the eastern border of the spray area in 1954.

emerged in the 2 weeks before spraying in 1956 (Fig. 4C). The numbers were above pre-spray numbers in 1958–60 and in 1962; in 1961 they dropped to a level about that of the low figure of 1957. Excessive flooding in May 1961 may have contributed to the low number of that year by reducing the populations of species which normally emerge in the early season.

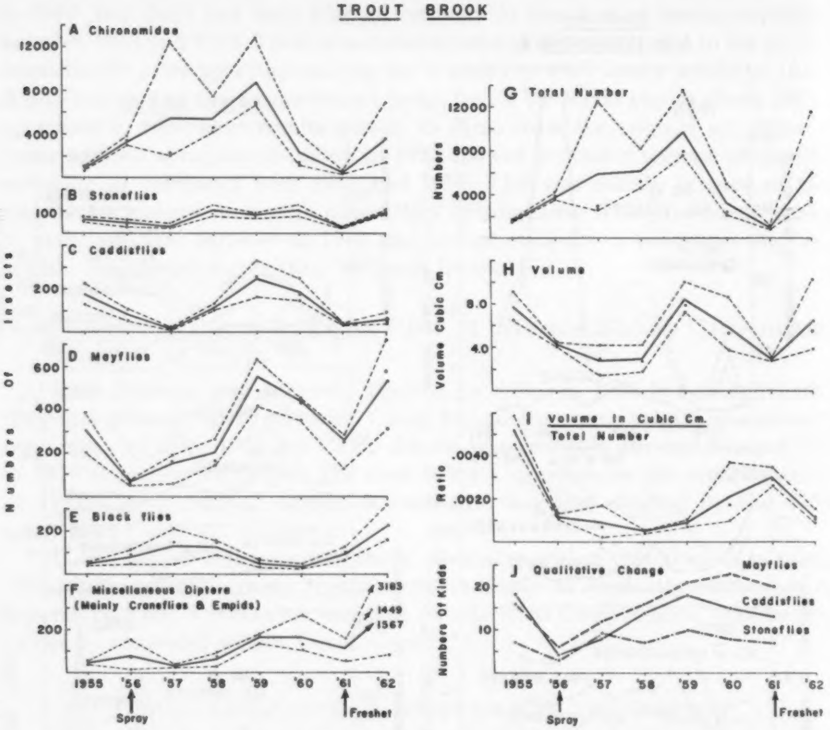

FIG. 10. A–F. Comparative annual numbers (60-day equivalents of actual numbers taken by two yard-square cage-traps operating for six 24-hr periods a week in 1955 and three such traps operating 5 days a week in other years from the beginning of June to the first week of September) of emerging insects of various groups for Trout Brook (T) for pre-spray year 1955, spray year 1956, and post-spray years 1957–62. G–J. Comparative annual data on total numbers and volumes of insects, average size, and estimated numbers of species for Trout Brook, 1955–62.

## CADDISFLIES (FIG. 10C)

The numbers of caddisflies were lowest in 1957, the year after spraying. Two influences evidently account for the higher numbers in the spray year than in the following year. Firstly, figures for 1956 include the individuals which emerged in the 2 weeks before spraying. Secondly, a large number emerged after spraying, nearly all of species of case-bearing Rhyacophilidae (Glossosomatinae); these are presumed to have been in the pupal stage at the time of spraying and, therefore, relatively unsusceptible to DDT. In 1957 these species were poorly represented and the numbers of all caddisflies (Fig. 4D, 10C) were low. The numbers were highest in 1959; they remained high in 1960 but in 1961 were lower than in 1956 but not as low as in 1957. Extremely high floods in late May of 1961 probably contributed to the low numbers in that year. In 1962 there was a slight rise in numbers.

### MAYFLIES (FIG. 10D)

The numbers of mayflies were lowest in 1956, the spray year. Exclusion of the individuals which emerged in the 2 weeks before spraying would have resulted in a still lower point for that year. Through 1957 to 1959 there was a continual rise in numbers; a decline in 1960 and a further decline in 1961 brought the numbers near those of the pre-spray year; in 1962 they were about the same as in 1959. This example shows clearly the greater disparity between the numbers for the different cage-traps when the numbers were high than when they were low, a reflection probably of differences in conditions of the bottom. The mayflies are in general herbivores (Muttkowski and Smith, 1929), and their increase in the years after spraying to higher than pre-spray numbers may have resulted from the sudden post-spray increase in food in the form of algae and the relative scarcity of predators (stoneflies, caddisflies).

### BLACK FLIES (FIG. 10E)

Changes in numbers of black flies resembled those of the chironomids more than any other group. Black flies are extremely sensitive to DDT in the larval stages (Jamnback and Collins, 1955; Jamnback and Eabry, 1962). Large numbers emerged early in the season of 1956, before the spraying was done, and the total for 1956 was greater than for the pre-spray year 1955 (Fig. 4B). The increased numbers in 1957 may represent progeny of ovipositing females from adjacent streams not affected by the 1956 spray, as discussed earlier. The females of some black fly species are known to disperse widely. The numbers from the cages varied widely in some years but were consistently low in 1959 and 1960, when caddisflies and stoneflies were abundant. The numbers of black flies were high in two of the three cages in 1957 and 1958 and again in 1961. None of the other groups of insects exhibited a comparable rise in 1961. In 1962 the black flies showed an even greater increase in numbers in the samples. The habit of aggregation of the larvae makes representative sampling difficult.

### MISCELLANEOUS DIPTERA (FIG. 10F)

The predominant forms in this group included species of *Antocha* (Tipulidae) and Empidae. Some Tipulidae are predatory, feeding on aquatic insect larvae (Muttkowski and Smith, 1929). Some Empidae are known to be predacious as adults, feeding on adult chironomids and small mayflies; their larvae eat larval stages of aquatic insects. By 1959 these insects were notably more abundant than in the pre-spray year, as were mayflies and chironomids. They remained well above the pre-spray numbers in 1960 and 1961, when other insects except black flies decreased in numbers. In 1962 they increased markedly in numbers. Their numbers varied widely between the different cage-traps in some years, one cage being high even in 1956, the spray year. There was also evidence of aggregation similar to that of the black flies.

### TOTAL NUMBERS (FIG. 10G)

The curves of total numbers of all aquatic insects followed closely those of the chironomids, because the latter generally contributed a high percentage of the totals.

### TOTAL VOLUMES (FIG. 10H)

Total volumes corresponded fairly well with the numbers of the larger insects such as caddisflies; there was a peak in 1959, the third year after spraying. Volumes were also influenced appreciably by the numbers of chironomids or other small insects when these were abundant. The volume was low in the spray year 1956, also in 1957, and again in 1961. Higher values were attained in 1958–60 and again in 1962. The 1961 low was associated with extreme flooding in that year, but intrinsic cyclic fluctuation of insect populations might also be involved.

### RATIO OF VOLUMES TO NUMBERS (FIG. 10I)

These ratios, indicating the average sizes of the individuals in the samples, should be considered along with Fig. 10G, H. When the ratio was low the volume was made up mostly of small insects, such as chironomids, black flies, and the small species of mayflies. Thus in 1956–59 and in 1962 small insects predominated, whereas in 1955, 1960, and 1961, when the ratio was higher, larger insects contributed more to the bulk.

### NUMBER OF KINDS (SPECIES) (FIG. 10J)

An attempt was made to list the numbers of species in the three major groups, stone flies, mayflies, and caddisflies, for 7 of the 8 years under consideration. Because of the technical nature of this work, it was impractical to complete the list in the time available. Many of the species, however, were recognizable and these were counted. For some closely related species, the group to which they belong was recognized and more than one species were treated as a unit. All stations were treated similarly and comparable results obtained. The counts were based on an examination of the samples from each year's collections; they represent the species recognized in the samples and not necessarily all that were taken. In some cases a species was represented by only one or two individuals in the samples of that year. This resulted in variable numbers of species being encountered even in later years of the survey when, presumably, the actual number should approach a constant value.

The smallest number of species for each group was represented in samples taken in the spray year, 1956, even though the sampling included 2 pre-spray weeks in that year. The mayflies and caddisflies showed this particularly, the stoneflies not so clearly because of the smaller number of species involved. In the two former groups the low point in the spray year was pronounced, and numbers of species near those of the pre-spray year were reached in 1958 by the mayflies and in 1959 by the caddisflies. The low points in all these qualitative curves occurred in the spray year even though the emergence of stoneflies and caddisflies had not yet reached their lowest numbers (Fig. 10B, C). It is significant that the severe flooding in 1961 did not reduce the numbers of species to near 1956 levels as with numbers and volumes in the populations.

It is not possible to conclude whether the increase in number of species in the years after spraying represented an increase from sparse residual numbers or whether there was immigration from other parts of the river system not affected by spray. There is some evidence from the small tributary of the Sevogle (Station SS, Appendix II) that headwaters sections of streams may be less affected by spray than an intermediate section (Station S, Appendix IV). It is possible that there was some immigration from upstream sections in Trout Brook by either immature stages or ovipositing females. Also, since Trout Brook empties into the main Northwest Miramichi, which was not sprayed, some species of insects may have migrated into Trout Brook from this source. The habit of caddisflies of flying upstream and ovipositing has been investigated by Roos (1957), who showed it to be an important factor in upstream repopulation.

## ANNUAL EMERGENCE OF MAJOR GROUPS AT OTHER STATIONS

The streams on which other stations were maintained for more than 1 year (Millstream Brook, North Branch of the Sevogle River, main Northwest Miramichi) are all parts of the Northwest Miramichi system and are all larger than Trout Brook at the station sites (Fig. 1). Millstream Brook (Station M) was used as a control, as described earlier.

The volume of all aquatic insects emerging at the control station averaged higher than at the sprayed station on the North Branch of the Sevogle (Station S) in every year and was also higher than at those (Camp Adams and Camp 42) on the Northwest Miramichi (Fig. 12). The numbers of insects emerging at the control station varied widely, not only from cage to cage but from year to year (Fig. 12), as did the figures for corresponding volumes.

Variable annual results for the control station would be expected as a result of differences in such factors as flooding, weather, and available food. Other variables are changing stream bottoms and exact cage positions. As mentioned earlier, Millstream Brook had less continuous rapids at the station site and the bottom was less uniform than at the Sevogle and Northwest Miramichi stations. The bottom under some of the cages was more silted or sandy than for others, causing embedding of stones and thus reducing the numbers of some groups of insects and increasing others. Another factor possibly affecting results for this station was the spraying. In 1957, although the plan called for exempting all of the Millstream Brook down to the station site, spraying was carried out over the lower reaches to a point within a quarter mile of the cages and it is not known whether the spray pattern was accurate enough to prevent some of the spray reaching the station site.

All the streams discussed below, except Millstream Brook, were included in the 1954 spray area and were seriously affected by spraying that year. The

Camp Adams site may have been affected again by adjacent spraying in 1956 (Fig. 1). The Sevogle site was sprayed for a second and third time in 1957 and 1958.

### MAYFLIES (FIG. 11)

Mayfly numbers at Millstream remained fairly constant from 1955 to 1958 with somewhat higher numbers in 1956 than in the other years. At the Sevogle (Station S) they were lower in 1955, the first post-spray year, than at Millstream the same year. In 1956 the numbers were nearly six times those of the previous year, and more than twice as great as at the control. In 1957 and 1958 they were sharply lowered, however, as the area was sprayed again in both years. In 1962, when the station was again sampled, the numbers of these insects were near the 1956 level, showing good recovery numerically. At Camp 42, sprayed in 1954 only, the numbers were rather high in 1957 when first sampled; in the subsequent years 1958–61 they maintained a plateau at a still higher level. More variation was shown at the Camp Adams station; numbers were roughly comparable to those of the control in 1959 and more than double this figure in 1960, 1961, and 1962.

### STONEFLIES (FIG. 11)

The Plecoptera showed considerable fluctuation in numbers at the control station. At the Sevogle a gradual decline in their numbers was evident after the 1954 and 1957 sprayings. The numbers were lowest in 1958, the year of the third spraying. The 1962 sampling showed larger numbers than in 1958 but gave evidence of only partial recovery as compared with the control. In 1955, the year after the first spraying, those emerging consisted almost entirely of small stoneflies of the genera *Nemoura* and *Leuctra*, mainly the latter. The numbers of these declined markedly in 1956, however, although no spraying was done in that year. At the Camp 42 station, the numbers of stoneflies were low in 1957 and 1958, the 3rd and 4th years after spraying. They reached a high in 1959 and dropped to less than two-thirds of the 1959 numbers in 1960 and 1961. At Camp Adams the numbers were high in 1959, 1961, and 1962 but in 1960 were only about a quarter as great.

### CADDISFLIES (FIG. 11)

The Trichoptera seem to have been more severely affected by the spraying than other groups. In the larger streams they were more affected than in the smaller Trout Brook or in the small tributary of the Sevogle River (Station SS, operated in 1955 only, Appendix II). Despite the gradual drop in numbers of caddisflies at the control site from 1955 to 1958, the consistently small numbers taken from the North Branch of the Sevogle through these same years are in sharp contrast, there being only a slight increase in 1962. At Camp 42 their numbers were low in 1957 and 1958, as at the Sevogle, but by 1960 they increased to a level about that of the control stream in 1958; this indicates a slower recovery than for the mayflies and stoneflies. The numbers of caddisflies at Camp 42 reached the level of those in the control in the 5th year after spraying. The mayflies showed about the same degree of recovery in 3 years and the stoneflies in 5 years. At Camp Adams the situation with respect to caddisflies was very different from that at Camp 42. The numbers were comparable to those in the control stream in 1959, a year earlier than at Camp 42. However, the large numbers emerging in one cage in 1959 were nearly all of one small species whose larvae were conspicuously aggregated. In 1960 and 1961 the numbers of caddisflies were markedly reduced at Camp Adams, being only about a quarter of those at Camp 42 in the same years. There was a slight increase in 1962.

It is difficult to clarify the apparently anomalous difference between caddisfly numbers at Camp Adams and Camp 42. The lower numbers at Camp 42 may have to do with the fact that the Camp Adams station was farther down the river, nearer the lower limit of 1954 spraying, and may have been repopulated by upstream migration earlier. The effect of spraying in the vicinity of Camp Adams in 1956 and below it in 1957 (Fig. 1) may also be involved. Also the river bottom at Camp Adams was more impregnated with silt and bark than at Camp 42 due to stripping of surrounding hills of timber.

### BLACK FLIES (FIG. 11)

The black flies were highly variable in numbers at these stations. The averages at the Camp 42 station do, however, show a general rising trend through 1957 to 1961 except for the slight drop in 1959. Numbers were low in all years at the Millstream Brook control station.

### CHIRONOMIDS (FIG. 11)

The Chironomidae showed great variability in numbers at the control and other locations when averages, and still greater variability when individual cage-trap results, are considered. The latter variation is considered to be mainly a result of differences in bottom. An anomalous feature for this group is the small numbers observed at the Millstream Brook and Sevogle stations in 1955 and at Camp 42 and Camp Adams in 1960 and 1961. In the intervening years, including spray years at the Sevogle, the numbers, in some cage-traps at least, were very high. Very large numbers emerged in one cage of the Sevogle series in 1956, 2 years after spraying and in one of the cages of the Camp 42 series in 1957, 3 years after spraying. Also, large numbers were noted particularly at Trout Brook in the latter half of the summer of 1956 and during the following 3 years (Fig. 7). Some of these high populations may be associated with reduction in the numbers of predatory insects which utilize the chironomids as food.

### MISCELLANEOUS DIPTERA (FIG. 11)

The predominant groups in this category were Tipulidae and Empidae. The variation in their average numbers was small at Millstream Brook. There

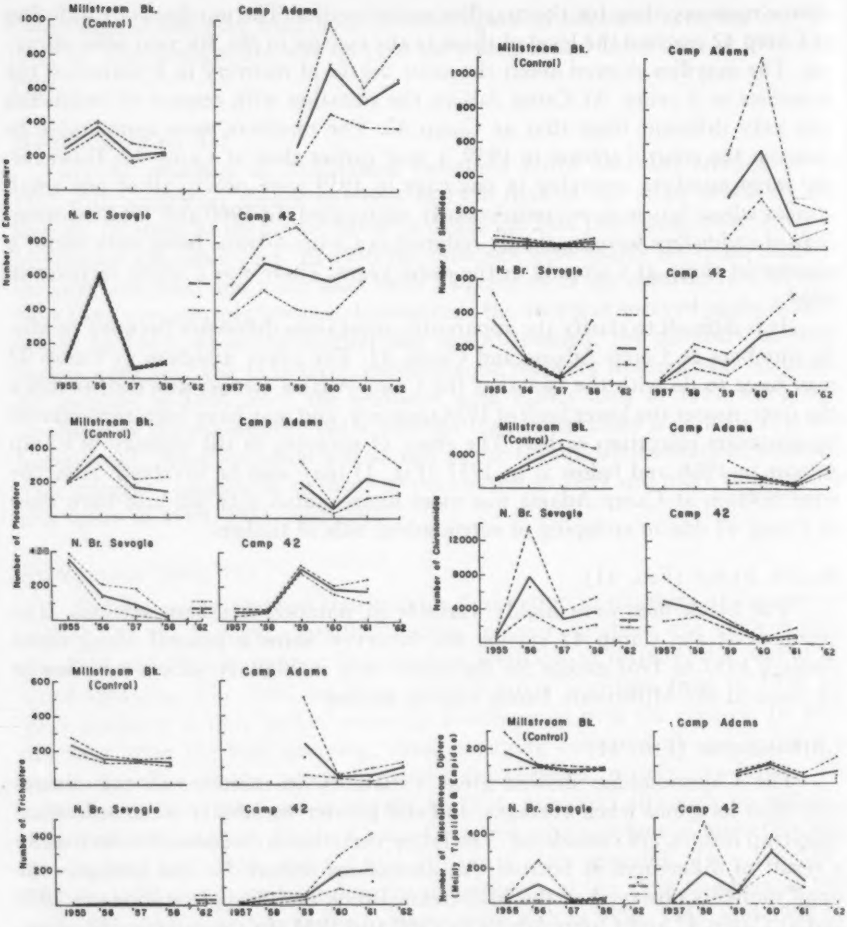

FIG. 11. Numbers of various groups of insects emerging annually at various stations (for further explanation see Fig. 10).

were, however, nearly twice as many of these flies emerging at this station in 1955 as in 1956, and the numbers were maintained at the lower level through 1957 and 1958. At the Sevogle, on the other hand, the numbers were low in 1955, the year after spraying. They increased to a point higher than the control numbers in 1956, then dropped to low figures in 1957 and 1958 with the second and third sprayings. At Camp 42 the numbers were low in 1957 but greatly increased in 1958–61; they were highest in 1960 and comparatively low in 1959. Camp Adams figures, on the other hand, show little variation in the numbers during the years 1959–62.

### TOTAL NUMBERS (FIG. 12)

Total numbers of emerging insects followed those for the chironomids because of the predominance of this group (see Fig. 11, 12).

### TOTAL VOLUMES (FIG. 12)

There was considerable variation in the values of this measure at the control station, but the averages were always higher than at any of the other three stations. The only year when the average volume of Sevogle insects approached that of the lowest figure for the control was in 1956, the second year after spraying, when mayflies were particularly numerous. The lowest volume for the Sevogle was in 1958, the year in which the station was sprayed for the third time. The numbers, however, were higher in 1958 than in 1957, but the insects were nearly all small in 1958 (Fig. 12). By 1962 the volume was still below that of 1955 and 1956 although no spraying had affected this location after 1958. The volumes for Camp 42 were lower than for the control in 1957 and 1958, increased slightly in 1959 and 1960, but decreased in 1961. The volumes for Camp Adams were somewhat lower than those for Camp 42 for corresponding years.

### RATIO OF VOLUME TO NUMBERS (FIG. 12)

These ratios, indicating the average sizes of the individuals making up the samples, should be considered in conjunction with Fig. 12A, B. At the Millstream Brook (control) the average size changed markedly from a high in 1955 to a low in 1957, with a slight rise in 1958. For the Sevogle, average size was always well below that for the control station in the same year. It was highest in 1955, the year after spraying, mainly because of the presence of large numbers of stoneflies (*Nemoura*, *Leuctra*) and mayflies (*Iron*, *Ephemerella*); these are larger insects which presumably survived the 1954 spraying mostly in the egg stage. In 1956 average size was low mainly because of large numbers of small chironomids. The lowest ratio was for 1958, the year of the third spraying, when emergence was predominantly of small insects. At Camp 42 average size was small in 1957 and 1958, and increasingly larger in the 3 following years. This reflects increasing numbers of larger insects, mainly stoneflies and caddisflies, and decreasing numbers of chironomids. It seems likely that utilization of chironomids as food by these carnivorous insects underlies these changes. At Camp Adams there was also an increase in average size, particularly in 1961, but in this year caddisflies were much scarcer than in 1959 whereas stoneflies were numerous. Chironomids were at their lowest numbers in 1961 at this station. The decrease in average size for Camp Adams samples in 1962 reflected the increased number of chironomids after extreme flood conditions in 1961; similar increases occurred at other stations.

### NUMBER OF KINDS (FIG. 12)

The numbers of species of mayflies, stoneflies, and caddisflies for the Millstream control were nearly constant in 1955 and 1957; in 1956, more species

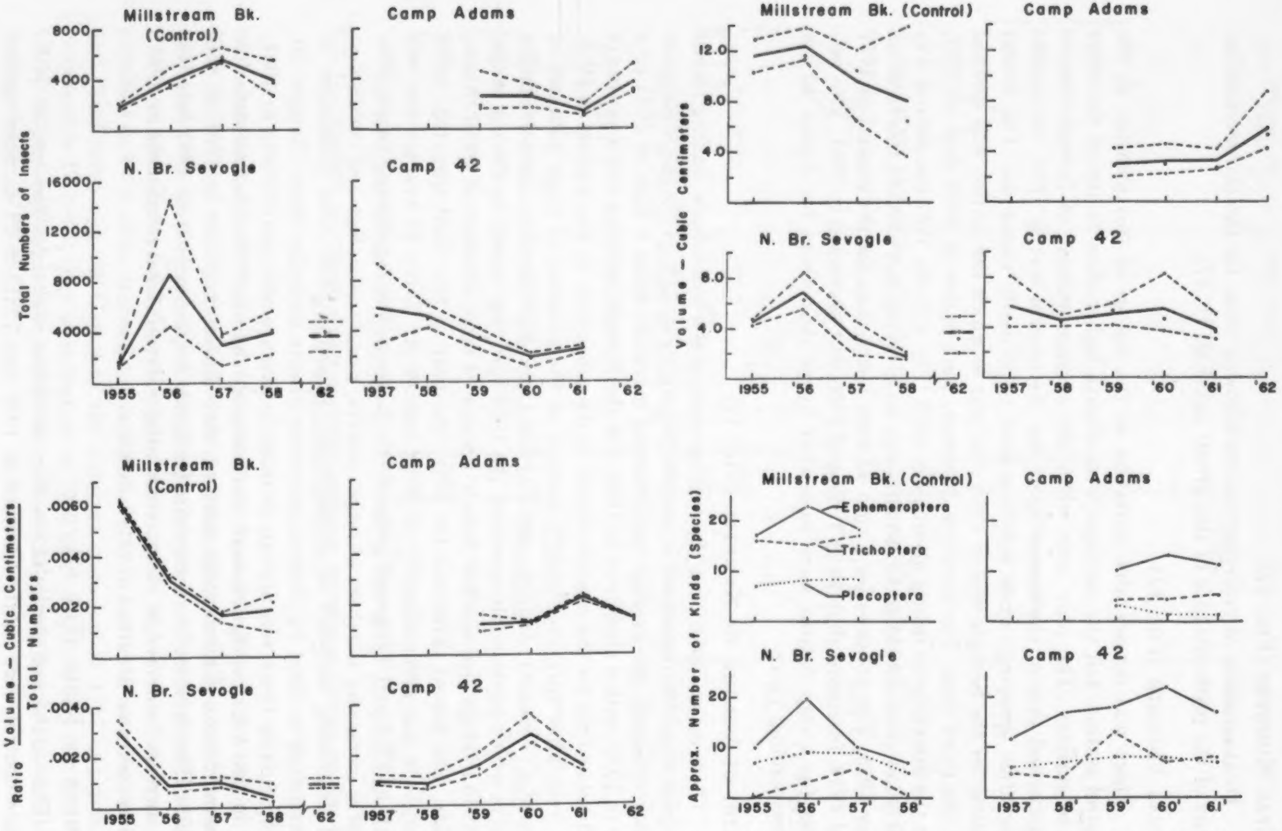

FIG. 12. Numbers of all insects emerging annually at various stations, volumes, ratios of volume to corresponding number, and estimated numbers of species of mayflies, stoneflies, and caddisflies (for further explanation see Fig. 10 and 11).

of mayflies were taken than in the other 2 years. There was a high total number of mayflies in samples of 1956 compared with other years, so that more species had a better chance of being taken. This finding should not be interpreted as indicating an increase in number of species at the station, but merely an increase in numbers represented in the samples. Similarly, the twofold increase in number of species of mayflies at the Sevogle in 1956 as compared with 1955 coincided with a larger number of these insects in the samples of the latter year. With spraying repeated in 1957 and 1958 the number of kinds in the samples was again down to the 1955 level in 1957 and lower in 1958. Caddisflies showed the most extreme change. None emerged in the three cages at the Sevogle in 1955. In 1956 three species were represented by small numbers, and in 1957 six species, showing a slow recovery. In 1958 the number of species was reduced to two which had survived the 1957 and 1958 sprayings. At the Camp 42 station the number of kinds of caddisflies was low in 1957 as compared with the Millstream Brook control; in 1959, 13 species were identified, and in 1960 and 1961 there were seven and eight respectively. The number of species recorded in 1957 at Camp 42 is a measure of the diversity in the fauna 3 years after application of the spray in 1954. The number recorded at Camp Adams in 1959 is a measure of the diversity in the same groups 5 years after the original spraying with a probable additional effect of spraying in the vicinity of the station in 1956.

## GENERAL DISCUSSION AND CONCLUSIONS

### INSECT FLUCTUATIONS WITHOUT SPRAYING INTERFERENCE

The normal course of emergence of the main insect groups through the season in the Northwest Miramichi area is suggested by the results of sampling in 1955 at Station T, Trout Brook (Fig. 4, 7), and from 1955 to 1958 at Station M, Millstream Brook (Fig. 5, Appendix III). The data for Station T show higher production of insects (chironomids not included) in the year before spraying than in those immediately after. Although details of day-to-day fluctuations in emergence of individual species are masked because the counts are totalled by major groups, some interesting features are apparent. For example, at both places the emergence of mayflies, stoneflies, and caddisflies was minimal at the start and end of observations for the season, and reached maximal daily levels around midsummer. Considerable variation within groups may occur for unknown reasons from one year to another, as indicated by the high production of black flies at Station T before spraying in 1956 as compared to the low emergence in the same early part of 1955.

Unusual natural occurrences may have severe effects on aquatic insect production, as shown by the extreme flooding conditions caused by a near-record freshet in May 1961. No unsprayed control stations were operated that year but marked effects of the freshet in cutting down production of three major insect groups for periods of a few days to several weeks is evident for stations T (Trout Brook) and N (Camp 42), where recovery from spraying was well advanced by 1961 (Fig. 4, 6).

## SHORT-TERM EFFECTS OF SPRAYING

With ½ lb DDT/acre used in forest spraying, reduction in numbers of the larval stages of aquatic insects ranged to 95% or even higher in local situations.

Trout Brook in 1956 and the North Branch of the Sevogle in 1957 and 1958 show the effect on emergence of aquatic insects in the year of spraying (Fig. 4, 7, 8). Figure 8 shows populations still recovering from an earlier spraying. The virtual cessation of emergence for up to 6 weeks after spraying occurred at a time of the year when large numbers of individuals of early summer species are normally emerging (Fig. 4, 7).

In a separate 1961 project on the Molus River, Kent County, N.B., to be published later, DDT was sprayed experimentally at ¼ lb/acre. Foot-square bottom sampling (Surber, 1937), before and after spraying, showed up to 97% reduction of bottom fauna as compared to actual increases in numbers during the same period in a nearby unsprayed control stream. Screens of 24-mesh copper (0.71-mm apertures), set to collect drifting spray casualties, took a heavy load of insects within an hour of spraying and the catch was heavy for several hours. This supports the conclusion that the 1956 drop in emergence at Trout Brook was a direct result of spraying DDT at ½ lb/acre.

As mentioned earlier, one of the visible results of DDT spraying in some of the more seriously affected parts of the streams was a conspicuous growth of various kinds of filamentous algae on the stones, forming a virtual mat of a brownish or greenish-brown colour. Many of the insects, including species of chironomids, mayflies, and some stoneflies, are known to be vegetarian (Muttkowski and Smith, 1929) and consequently much food should be available to such insects. Low numbers of predatory species, including some stoneflies and caddisflies, which occurred at the same time, would mean that large populations of the primary feeders could be supported and more than pre-spray numbers of these might be expected. Thus chironomids were present and emerging in large numbers in the late summer of the spray year and in the following year.

Gorham (1961) reports on an investigation in the State of Maine, adjacent to the New Brunswick border, where in 1958 forests were sprayed with a dosage of 1 lb DDT-in-oil/acre. Using the Surber technique he concluded that recovery was good in some groups and fair in others, including caddisflies, by August 1959. Gorham states, "It is very unlikely that the 1958 spruce budworm control campaign caused or will cause the extinction of any species in the spray area or that it had any significant long-term or permanent deleterious effects on the populations of riffle insects." His conclusions are supported by the present paper as to the effects on major categories of insects. He has, for instance, demonstrated a rise in the number of chironomids above pre-spray numbers in late summer of the spray year, which agrees with the present findings but not with those of Webb and Macdonald (MS, 1958); Surber sampling by these workers indicated that chironomids were usually more abundant in unsprayed than in sprayed streams. Gorham's conclusion that it is unlikely that any species were eliminated is premature, since his identifications were not made to the species level.

### RECOVERY IN THE SPRAY YEAR

Obviously the decreased emergence in the first weeks after spraying was due to a reduction in numbers of larvae and nymphs, the few insects which continued to emerge being mostly from viable pupae. Glossosomatin caddisflies, for instance, emerged in rather large numbers at Trout Brook after spraying in 1956 (Fig. 4D). These case-making types survived; they were probably pupae within sealed cocoons at spray time. The few chironomids which emerged in this period were also probably pupae when the spray was applied. An occasional mayfly and stonefly also emerged throughout this period, presumably having survived because of comparatively low susceptibility to DDT. The great increase in numbers emerging began, however, about 4 weeks after spraying at Trout Brook (Fig. 7) and after a somewhat longer interval at the North Branch of the Sevogle in 1957 and 1958 (Fig. 8). These increases were mostly in chironomids, but black flies and other Diptera and a few caddisflies were also involved. The numbers of chironomids emerging in cage-traps of the Trout Brook series in the late summer peak of 1956 were higher than those of the pre-spray year (Fig. 10A). This large chironomid population is presumed to be mainly of the second-generation individuals of bivoltine species which survived spraying in June in the egg stage.

An observation made at Gill Brook a month after spraying in 1954 is pertinent in this connection. Stones picked from the bottom in rapids were found to be densely populated with recently hatched chironomid larvae, estimated to number about 700 per foot$^2$. It is possible that these were progeny of ovipositing females that immigrated from some unaffected stream, but this is improbable because of Gill Brook's distance from the spray border and the limited flight range of chironomid adults. It is more probable that they survived spraying in the egg stage.

A few specimens of two species of caddisflies of the family Limnephilidae, *Neophylax* sp. and *Pycnopsyche* sp., emerged at the end of August 1956 at Trout Brook. The species are both relatively large insects and their larvae live in cases. Both normally pass through most of the summer as prepupae in diapause, believed to afford protection against DDT.

There was, therefore, almost a complete absence of well-grown active larvae and nymphs for several weeks after spraying. Presumably viable eggs and pupae and some newly hatched individuals of a few species were present during this period. More nymphs and larvae became evident in July with the hatching of more eggs. This was followed by the emergence, in July, August, or September, of large numbers of insects (mostly chironomids). The populations of these were sometimes so large that numerical recovery occurred in the same season as the spraying. The bottom fauna was, however, deficient in bulk and variety.

### RECOVERY IN SUBSEQUENT YEARS

In the year after spraying, insects of some groups, including mayflies, emerged in such large numbers that it was evident that they had not reached these numbers by reproduction from a few survivors of the spray. For instance, three species of mayflies, *Iron pleuralis*, and two species of *Ephemerella*, emerged in considerable numbers at the Sevogle station in 1955, the year after spraying (Fig. 5). These are spring or early summer species, which oviposited before spraying in mid-June. *Iron pleuralis* lays eggs which remain in diapause for 3 months (Ide, 1935) and presumably these survived the spraying in 1954, hatched in numbers in the fall of 1954, and emerged in 1955. The early species of *Ephemerella* may be in the same category but in addition some of these have been observed in other years to be relatively tolerant of DDT, as demonstrated by their occurrence in post-spray Surber samples. Hitchcock (1960) mentions that he has found these insects to be somewhat tolerant of spray in the nymphal stages.

Some stoneflies also appeared in large numbers at the North Branch of the Sevogle in 1955, the year after spraying (Fig. 5). These were almost all species of *Nemoura*, early emerging stoneflies, and species of *Leuctra*, some of which are early and some mid- to late-summer species. Most are presumed to have survived the spraying in the egg stage but some may have survived as nymphs. The particularly large numbers of stoneflies emerging at Station SS on the small tributary stream of the North Branch of the Sevogle in the year after spraying (Appendix II) were mainly species of *Leuctra*.

The above are examples of survival of large populations because of tolerance to DDT in some stage of the life cycle. These particular examples are organisms which require a year to complete the life cycle so that large numbers the year after spraying imply survival in numbers and not an increase through multiplication. Despite the above-mentioned species emerging in appreciable numbers the year after spraying, the numbers of caddisflies and stoneflies in Trout Brook were lower in the year after spraying than in the spray year (Fig. 4C and D, 10). But the number of emerging mayflies was lowest in the spray year although the totals included an appreciable number emerging before the spraying. Also, the number of kinds or species was lowest in the spray year for the groups considered in this way (Fig. 10).

The result of the spraying on the insects of the streams may be summed up as follows: spraying was followed by almost 100% reduction in numbers of most insects which were in active stages of the life cycle, e.g. feeding larvae or nymphs. Species in tolerant stages, such as eggs, pupae or diapausing larvae, survived in appreciable numbers, but the total insect population in immediate post-spray years was mainly composed of a few species representing these survivors. Other species, because of greater vulnerability at the particular period when spraying was carried out, were greatly reduced or even eliminated from the local area. These species only increased to a significant element of the population after a lapse of time which allowed them to multiply either from residual small numbers or from natural reintroductions from less affected waters.

Most groups of insects appear to have been reduced in numbers of individuals immediately after spraying. In general, this was followed by an increase to numbers exceeding pre-spray numbers 2 or 3 years after spraying and then a drop to near or below pre-spray numbers eventually for some groups. Figure 10 shows this for Trout Brook. The different times involved in phases of recovery may be the result of a number of influences including: food supply; whether the insects are mainly herbivorous or carnivorous; possession of DDT-tolerant stages in the life cycle; and rate of multiplication, which depends on whether the insects are small with a short life cycle, or large and slow-growing, and whether they are bivoltine or univoltine. Some of the large stoneflies, e.g. *Acroneuria* species, require 2 years to complete the life cycle and recovery in numbers of these by reproduction from survivors was comparatively slow.

After spraying in 1956, mayflies in Trout Brook, particularly some of the small Baetinae, increased to large numbers in 1957 and 1958 and a high in 1959, and then declined until 1961 (Fig. 4A, 10D). Stoneflies did not fluctuate as much as the mayflies and reached their greatest numbers somewhat later. Caddisflies, particularly the larger species, lagged still more. Black flies showed an increase in some cages similar to that of the chironomids, with a rapid rise in numbers after spraying (Fig. 10). They decreased earlier than chironomids, but increased in 1961 as well as in 1962. The "miscellaneous Diptera," mainly Tipulidae and Empidae, are mostly predacious. Some of the empids prey as larvae on the aquatic larval stages of insects and also as adults on adult chironomids and small mayflies. The numbers of miscellaneous Diptera (Fig. 10), although variable, showed a general trend in which their high numbers lagged behind those of their prey in a manner which has been observed in many predator–prey relations.

The curve for total numbers of insects for Trout Brook (Fig. 10G) follows closely that for chironomids because of the predominance of this group. The curve for corresponding volumes (Fig. 10H) does not follow that for numbers but in general shows a drop in bulk of insects through spray and immediate post-spray years; this is mainly because of large chironomid numbers. The ratio curve, indicating average size, shows a general rise from 1958 to 1961 in association with the increasing numbers of large insects in the population. A drop in 1962 was associated with a greater increase in numbers of smaller than of larger kinds after the 1961 freshet.

Respraying of the Sevogle in 1957 and 1958 interrupted the rehabilitation process there, but the increase in larger species at Camp 42, as shown for the caddisflies (Fig. 11), would indicate that the early rise in numbers of chironomids was followed within several years by a rise in other groups. Recovery at Camp 42 can probably be assumed to have paralleled that of the North Branch of the Sevogle through 1955 and 1956. Thus the two together give a continuous record of the probable pattern of recovery in insect populations for a river which was extremely affected by spraying in 1954.

Camp Adams, 10 miles downstream from Camp 42, shows some significant differences from the latter. Some of these may be a result of its closer proximity to the downstream limit of 1954 spraying and therefore to a less depleted fauna as a source of immigrants. The variety of caddisflies at Camp Adams in 1959 was considerably greater than at Camp 42 the same year, particularly in the presence of a number of Hydropsychidae, absent at that time from Camp 42. This was in spite of a 1956 spraying that possibly affected Camp Adams but not Camp 42.

Evidently numbers alone are not a valid criterion of recovery of aquatic insects in the streams after spraying. Volume gives a somewhat better indication, but even this is not completely satisfactory. If the volume is large merely because of high numbers of very small insects, it cannot meet the feeding requirements of all kinds and sizes of fish. A large volume made up of a considerable proportion of larger insects, as in pre-spray conditions, can provide satisfactory food for insect-eating fishes of all sizes, and should therefore be a better indicator of carrying capacity than numbers or volume alone.

From the standpoint of permanent damage to streams by spraying, and particularly by repeated spraying, the recovery in variety of aquatic insects would seem to be a most important indicator. Such recovery for mayflies, stoneflies, and caddisflies is shown for Trout Brook in Fig. 10J and for the Sevogle and the Northwest Miramichi in Fig. 12. From these it would appear that fairly good recovery of the three groups of insects took place, but that it was slower for caddisflies than for the other two groups. The Trout Brook station was not as severely affected as was the North Branch of the Sevogle or the Northwest Miramichi, perhaps because it was less remote from unsprayed areas. Such a situation has also been noted by Hitchcock (1960).

Summarizing, if numbers of insects were an adequate criterion, then at least some streams "recovered" in from 6 weeks to 2 months after being sprayed. But because of their small size these numerous insects are not satisfactory food for larger fish, although they are suitable for fry. When, however, the bulk of organisms and their individual size are taken as the criterion, recovery was much slower (Fig. 10I, 12). It then paralleled more closely the increase in number of species, eventually approaching the diverse fauna characteristic of the pre-spray condition (Fig. 10I, J). Present data indicate that this required up to 3 years for mayflies and 4 or more for caddisflies.

### BOTTOM FAUNA AND FISH FOOD

The salmon fry are mainly insectivorous and feed on small insects, including chironomids and small mayflies. In the overyearling stage higher percentages of larger insects are used (White, 1936; Hurley, MS, 1956; Keenleyside, 1967).

As insects are preferred food of young salmon in streams, any treatment which eliminated them, even if molluscs, annelids, and other lower invertebrates were to increase, would be deleterious. Allen (1941) has shown that young Atlantic salmon are selective in their feeding. Hurley (MS, 1956) found that molluscs (Physidae) were extensively eaten (up to 92.6% of stomach contents) by salmon parr in the Northwest Miramichi River in 1955. Here bottom sampling had shown that they formed a high percentage of the bottom fauna particularly in the latter part of the summer, in contrast with low numbers at locations outside the boundary of 1954 spraying. These molluscs were absent from the stomachs of salmon parr taken from areas outside the spray boundary in 1955 and from the stomachs of parr taken from all locations in the pre-spray year 1953. In the absence of aquatic insects normally utilized the young salmon did take the snails in this local situation but they are unusual components of their diet.

Since aquatic insects formed the largest component of the diet of young salmon before spraying (Keenleyside, 1967), changes in their numbers as a result of spraying had an effect on the diet and consequently on the population of the fishes. Detailed accounts of changes in the populations of young salmon in the Northwest Miramichi through the interval covered by this report are given by Kerswill (1967) and Elson (1967). In 1954, for instance, spraying resulted in the virtual elimination of salmon fry in the section of the Northwest Miramichi in which the Camp 42 and Camp Adams stations were located and a marked reduction in numbers of overyearling young salmon. The emaciated condition of survivors was indicative of poor feeding conditions.

Unfortunately intensive sampling of aquatic insects was not carried out on the Northwest Miramichi at this critical period nor for several years afterwards, because of inaccessibility of the area by road. However, it can be assumed that the immediate effect of spraying the Northwest Miramichi in 1954 was similar to that already described for the spraying of Trout Brook in 1956 and the Sevogle in 1957 and 1958. In all cases there was a high mortality among active stages of aquatic insects. One such case of the immediate effect of spraying was followed in detail on the Molus River in 1961 (see "Short-term Effects of Spraying"). Whether killing of the salmon fry was indirect, from feeding on poisoned insects, or by direct contact with DDT in the water, has not been satisfactorily determined.

For an interval after spraying there was a period during which very few insects were evident on the bottom. After this, newly hatched insects began to develop and eventually emerge, frequently in very large numbers late in the summer, as was described for both Trout Brook in 1956 and the Sevogle in 1957 and 1958. Most of these insects were very small species, predominantly chironomids. The salmon fry in the Northwest Miramichi did not survive this sequence of events in 1954 (Elson, 1967), but some overyearlings did, although some were in poor condition. The spawning in the fall of 1954 resulted in a particularly high population of fry according to sampling records made in the late summer of 1955 (Elson, 1967).

The abundance of chironomid adults at Trout Brook in 1956 and 1957 suggests that small larvae of chironomids, on which fry feed, were abundant when this large population of fry was feeding. In later years when these became overyearlings and larger, there was a higher proportion of larger insects, more suitable food for these larger fish. It would appear, therefore, that whereas the spray eliminated the fry that hatched in 1954, those that hatched in 1955 were probably provided with satisfactory quantities of suitable insects. Repeated spraying in the same summer or in consecutive years (Kerswill, 1967) would, however, probably produce very deleterious effects on the aquatic insect food of young salmon.

## Other Effects of DDT

Long-term records of Surber sampling on several streams, one of which, Ramsay Brook, was sprayed for the 7 consecutive years 1952–58 (Webb and Macdonald, MS, 1958; Macdonald, 1965), give a long and continuous record of spray effects on the bottom fauna. Some of the recovery in 1958 in Ramsay Brook was in populations of a small mayfly which was exceedingly abundant even after this history of spraying. This raised the question of the possible development of immunity to DDT in these insects, such as has been found for some species (Brown et al., 1963). Experiments started in summer 1965 at the Department of Zoology, University of Western Ontario, showed that mayfly nymphs in one species of the genus *Heptagenia* developed at least a 15-fold DDT-tolerance in areas of New Brunswick sprayed annually with DDT since 1956 with the exception of 1959 (C. D. Grant, personal communication).

The present studies do not include any examples of streams sprayed as many times as Ramsay Brook, the maximum being three (North Branch of the Sevogle). Consequently, the present work throws no light on the question of selection of DDT-resistant strains. But there are evidently cases where the spray caused little reduction in numbers because the insects were in the egg stage through the toxic period. Second generations of some of the small bivoltine mayflies appearing in late summer of the spray year (Fig. 4A) are presumed to be from eggs laid before spraying by the first generations. These are not considered to have been selected by the spray, although there may have been some selection against individuals which hatched soon after spraying when conditions were still toxic or against late individuals of the first generation exposed to spray.

## Sampling Problems

In this investigation organisms other than aquatic insects, such as molluscs and annelids, were not considered since the method did not provide a means of sampling them. This is a shortcoming in the method, as Webb and Macdonald (MS, 1958) and Webb et al. (MS, 1959) point out. However, it is only a minor drawback when the predominant position of aquatic insects in the natural food of salmonid fishes is considered. Webb and Macdonald also note that cage-traps sample the insects after they have left the stream bottom, and conclude that the samples are not a good index of available food. Fish food studies show, on the contrary, that most aquatic insects become more vulnerable to fish predation in their last nymphal instar, when they move from their normal protected situations into positions suitable for emergence, and that they are exceptionally vulnerable just before and during emergence. Hence emergence records are more closely related to feeding opportunities than are bottom samples.

Apart from that, the cage-trap method has two great advantages over bottom sampling. One feature is its continuity, which reflects the course of insect emergence, so that emergence records reflect the biological production of these animals better than bottom sampling. Some species complete their development in less than 3 weeks, e.g. some black flies; others take 2 or even 3 years, e.g. some stoneflies. The other advantage of the cage-trap is its greater efficiency in sampling minute insects such as the smallest chironomids, which are frequently very abundant and are utilized as food by salmon fry. Bottom sampling, however, may be a useful adjunct to cage-trapping in giving a comparative measure of the standing bottom crop of larger organisms.

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Appendices I–VI *follow*.

Appendix I. Annual emergence for Trout Brook (T), 1955–62. Annual numbers of insects emerging into two yard-square cage-traps operated for six 24-hr intervals per week in 1955 and three such cages operated for five 24-hr intervals per week in other years from approximately the beginning of June to the first week of September and made comparable by reducing all to 60-day equivalent values.

| | | Numbers | | | | | | | | Ratio vol. in *cc* |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Diptera | | | | | |
| Year | Cage-trap | Ephem. | Plec. | Trich. | Chiro. | Sim. | Misc. | Total | Volume in *cc* | Numbers |
| 1955 | 1 | 203 | 78 | 133 | 890 | 41 | 44 | 1389 | 6.4 | 0.0045 |
| | 2 | 384 | 53 | 228 | 735 | 58 | 41. | 1502 | 9.1 | 0.0059 |
| 1956 | 1 | 48 | 62 | 111 | 3005 | 90 | 150 | 3466 | 4.4 | 0.0013 |
| | 2 | 69 | 36 | 54 | 3430 | 111 | 69 | 3769 | 4.2 | 0.0011 |
| | 3 | 62 | 45 | 81 | 3860 | 45 | 15 | 4108 | 4.1 | 0.0010 |
| 1957 | 1 | 53 | 45 | 16 | 12400 | 43 | 22 | 12579 | 3.0 | 0.0002 |
| | 2 | 188 | 29 | 20 | 2090 | 148 | 28 | 2503 | 4.3 | 0.0017 |
| | 3 | 164 | 21 | 15 | 2200 | 208 | 33 | 2641 | 1.4 | 0.0007 |
| 1958 | 1 | 265 | 91 | 135 | 7550 | 85 | 76 | 8202 | 4.3 | 0.0005 |
| | 2 | 140 | 83 | 101 | 4260 | 152 | 23 | 4759 | 1.8 | 0.0004 |
| | 3 | 162 | 134 | 112 | 4630 | 145 | 88 | 5271 | 2.8 | 0.0005 |
| 1959 | 1 | 418 | 79 | 260 | 12800 | 30 | 176 | 13763 | 10.0 | 0.0007 |
| | 2 | 635 | 75 | 162 | 6640 | 51 | 134 | 7697 | 7.9 | 0.0010 |
| | 3 | 490 | 99 | 334 | 6780 | 61 | 169 | 7933 | 7.3 | 0.0009 |
| 1960 | 1 | 458 | 102 | 247 | 4130 | 25 | 123 | 5085 | 5.2 | 0.0010 |
| | 2 | 430 | 133 | 179 | 1300 | 43 | 266 | 2351 | 8.6 | 0.0037 |
| | 3 | 352 | 83 | 142 | 1500 | 29 | 100 | 2206 | 3.8 | 0.0017 |
| 1961 | 1 | 129 | 27 | 42 | 645 | 99 | 50 | 992 | 2.9 | 0.0029 |
| | 2 | 250 | 28 | 28 | 255 | 124 | 116 | 801 | 2.8 | 0.0035 |
| | 3 | 282 | 32 | 41 | 670 | 63 | 158 | 1246 | 3.2 | 0.0026 |
| 1962 | 1 | 720 | 93 | 104 | 7130 | 167 | 3183 | 11397 | 10.25 | 0.0009 |
| | 2 | 568 | 71 | 51 | 2364 | 339 | 90 | 3493 | 5.25 | 0.0015 |
| | 3 | 305 | 83 | 39 | 1540 | 167 | 1442 | 3576 | 3.91 | 0.0011 |

[a] Ephem. = Ephemeroptera (mayflies); Plec. = Plecoptera (stoneflies); Trich. = Trichoptera (caddisflies); Chiro. = Chironomidae; Sim. = Simuliidae (black flies); Misc. = miscellaneous Diptera (mainly Tipulidae and Empidae).

Appendix II. Sixty-day equivalent figures for two cage-traps at small tributary of North Branch of the Sevogle (SS) in 1955. (See Appendix I for further explanation.)

| | | Numbers | | | | | | | | Ratio vol. in *cc* |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Diptera | | | | | |
| Year | Cage-trap | Ephem. | Plec. | Trich. | Chiro. | Sim. | Misc. | Total | Volume in *cc* | Numbers |
| 1955 | 1 | 393 | 527 | 11 | 355 | 15 | 69 | 1370 | 6.8 | 0.0051 |
| | 2 | 275 | 500 | 1 | 640 | 5 | 23 | 1444 | 4.7 | 0.0031 |

APPENDIX III. Sixty-day equivalent figures for Millstream Brook (M) (control) cage-traps 1955–58. (See Appendix I for further explanation.)

| | | Numbers | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Diptera | | | | | Ratio vol. in *cc* |
| Year | Cage-trap | Ephem. | Plec. | Trich. | Chiro. | Sim. | Misc. | Total | Volume in *cc* | Numbers |
| 1955 | 1 | 282 | 214 | 278 | 968 | 55 | 288 | 2085 | 12.6 | 0.0061 |
| | 2 | 210 | 192 | 186 | 1110 | 22 | 66 | 1786 | 10.0 | 0.0063 |
| 1956 | 1 | 329 | 293 | 143 | 2557 | 9 | 87 | 3418 | 11.2 | 0.0033 |
| | 2 | 401 | 446 | 171 | 2371 | 38 | 99 | 3526 | 11.9 | 0.0032 |
| | 3 | 404 | 357 | 128 | 3625 | 35 | 92 | 4641 | 15.6 | 0.0028 |
| 1957 | 1 | 279 | 221 | 138 | 5610 | 5 | 94 | 6347 | 11.8 | 0.0018 |
| | 2 | 176 | 186 | 140 | 5100 | 3 | 59 | 5664 | 10.2 | 0.0018 |
| | 3 | 163 | 118 | 127 | 3910 | 10 | 64 | 4392 | 6.2 | 0.0014 |
| 1958 | 1 | 252 | 118 | 159 | 2110 | 33 | 82 | 2754 | 6.2 | 0.0022 |
| | 2 | 210 | 240 | 117 | 4730 | 15 | 85 | 5397 | 13.6 | 0.0025 |
| | 3 | 210 | 94 | 110 | 3180 | 51 | 70 | 3715 | 3.9 | 0.0010 |

APPENDIX IV. Sixty-day equivalents figures for North Branch of Sevogle (S) cage-traps 1955–58 and 1962. (See Appendix I for further explanation.)

| | | Numbers | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Diptera | | | | | Ratio vol. in *cc* |
| Year | Cage-trap | Ephem. | Plec. | Trich. | Chiro. | Sim. | Misc. | Total | Volume in *cc* | Numbers |
| 1955 | 3 | 99 | 383 | 0 | 975 | 213 | 8 | 1678 | 4.3 | 0.0026 |
| | 4 | 109 | 335 | 0 | 337 | 524 | 27 | 1332 | 4.7 | 0.0035 |
| 1956 | 3 | 601 | 214 | 4 | 13545 | 124 | 17 | 14505 | 8.4 | 0.0006 |
| | 4 | 586 | 139 | 2 | 6418 | 138 | 49 | 7332 | 6.3 | 0.0009 |
| | 5 | 613 | 75 | 2 | 3330 | 150 | 247 | 4417 | 5.5 | 0.0012 |
| 1957 | 3 | 52 | 186 | 3 | 3470 | 27 | 4 | 3742 | 4.5 | 0.0012 |
| | 4 | 68 | 80 | 4 | 3680 | 37 | 9 | 3878 | 3.2 | 0.0008 |
| | 5 | 54 | 22 | 5 | 1254 | 36 | 6 | 1377 | 1.8 | 0.0011 |
| 1958 | 3 | 86 | 28 | 0 | 2050 | 110 | 33 | 2307 | 2.0 | 0.0009 |
| | 4 | 77 | 22 | 2 | 5360 | 196 | 10 | 5667 | 1.4 | 0.0002 |
| | 5 | 100 | 6 | 3 | 3570 | 378 | 22 | 4079 | 1.8 | 0.0004 |
| | 6 | 74 | 32 | 5 | 2810 | 24 | 23 | 2968 | 1.6 | 0.0005 |
| | 7 | 121 | 31 | 1 | 3270 | 142 | 81 | 3646 | 2.6 | 0.0007 |
| | 8 | 77 | 21 | 3 | 2980 | 48 | 60 | 3189 | 1.5 | 0.0005 |
| 1962 | 3 | 482 | 116 | 26 | 3620 | 119 | 146 | 4509 | 3.2 | 0.0007 |
| | 4 | 821 | 57 | 54 | 2769 | 400 | 124 | 4225 | 4.8 | 0.0012 |
| | 5 | 404 | 58 | 15 | 1967 | 125 | 57 | 2626 | 2.0 | 0.0008 |

APPENDIX V. Sixty-day equivalent figures for Camp 42 (N) cage-traps 1957–61. (See Appendix I for further explanation.)

| | | Numbers | | | | | | | | Ratio vol. in *cc* |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Diptera | | | | | |
| Year | Cage-trap | Ephem. | Plec. | Trich. | Chiro. | Sim. | Misc. | Total | Volume in *cc* | Numbers |
| 1957 | 1 | 467 | 31 | 4 | 4580 | 16 | 13 | 5111 | 4.8 | 0.0009 |
| | 2 | 550 | 49 | 5 | 7920 | 10 | 10 | 8544 | 8.2 | 0.0009 |
| | 3 | 373 | 77 | 13 | 2475 | 26 | 19 | 2983 | 4.1 | 0.0013 |
| 1958 | 1 | 815 | 57 | 3 | 5004 | 193 | 13 | 6085 | 4.4 | 0.0007 |
| | 2 | 520 | 65 | 65 | 3060 | 92 | 475 | 4277 | 5.0 | 0.0012 |
| | 3 | 710 | 49 | 15 | 4110 | 234 | 54 | 5172 | 4.1 | 0.0008 |
| 1959 | 1 | 890 | 324 | 59 | 2650 | 176 | 63 | 4162 | 5.9 | 0.0014 |
| | 2 | 404 | 308 | 46 | 1574 | 52 | 68 | 2452 | 5.3 | 0.0021 |
| | 3 | 595 | 282 | 31 | 2290 | 124 | 80 | 3402 | 4.1 | 0.0012 |
| | 4 | 439 | 130 | 37 | 1638 | 79 | 81 | 2404 | 2.3 | 0.0010 |
| | 5 | 903 | 190 | 111 | 3000 | 122 | 572 | 4898 | 6.2 | 0.0013 |
| | 6 | 479 | 79 | 35 | 1300 | 123 | 173 | 2189 | 1.9 | 0.0009 |
| 1960 | 1 | 698 | 160 | 285 | 489 | 313 | 387 | 2332 | 8.2 | 0.0036 |
| | 2 | 380 | 189 | 90 | 243 | 356 | 315 | 1573 | 3.7 | 0.0024 |
| | 3 | 649 | 207 | 172 | 552 | 178 | 197 | 1955 | 4.7 | 0.0024 |
| | 4 | 930 | 336 | 37 | 857 | 440 | 51 | 2651 | 6.5 | 0.0025 |
| | 5 | 665 | 396 | 49 | 807 | 434 | 235 | 2586 | 6.7 | 0.0026 |
| | 6 | 1085 | 349 | 41 | 1545 | 175 | 55 | 3250 | 6.8 | 0.0021 |
| 1961 | 1 | 575 | 188 | 51 | 1860 | 37 | 96 | 2807 | 3.6 | 0.0013 |
| | 2 | 771 | 105 | 133 | 660 | 405 | 218 | 2292 | 3.0 | 0.0013 |
| | 3 | 627 | 244 | 412 | 750 | 527 | 96 | 2656 | 5.0 | 0.0019 |
| | 4 | 476 | 119 | 95 | 1055 | 130 | 99 | 1974 | 2.3 | 0.0012 |
| | 5 | 375 | 161 | 18 | 1330 | 198 | 64 | 2146 | 1.9 | 0.0009 |
| | 6 | 620 | 98 | 21 | 730 | 464 | 74 | 2007 | 2.5 | 0.0012 |

APPENDIX VI. Sixty-day equivalent figures for Camp Adams (NC) cage-traps 1959–62. (See Appendix I for further explanation.)

| | | Numbers | | | | | | | | Ratio vol. in *cc* |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Diptera | | | | | |
| Year | Cage-trap | Ephem. | Plec. | Trich. | Chiro. | Sim. | Misc. | Total | Volume in *cc* | Numbers |
| 1959 | 1 | 388 | 335 | 505 | 3170 | 55 | 48 | 4501 | 4.3 | 0.0009 |
| | 2 | 200 | 126 | 55 | 639 | 540 | 41 | 1601 | 1.9 | 0.0012 |
| | 3 | 159 | 178 | 156 | 912 | 287 | 74 | 1766 | 2.8 | 0.0016 |
| 1960 | 1 | 992 | 76 | 39 | 1135 | 1101 | 118 | 3461 | 4.6 | 0.0013 |
| | 2 | 745 | 62 | 52 | 1332 | 326 | 87 | 2604 | 3.0 | 0.0012 |
| | 3 | 457 | 25 | 38 | 774 | 284 | 120 | 1698 | 2.2 | 0.0013 |
| 1961 | 1 | 605 | 442 | 89 | 532 | 269 | 48 | 1985 | 4.2 | 0.0021 |
| | 2 | 380 | 155 | 14 | 452 | 73 | 12 | 1086 | 2.6 | 0.0024 |
| | 3 | 572 | 114 | 48 | 305 | 45 | 58 | 1142 | 2.7 | 0.0023 |
| 1962 | 1 | 652 | 143 | 61 | 2082 | 160 | 38 | 3136 | 4.6 | 0.0015 |
| | 2 | 875 | 305 | 130 | 3830 | 218 | 168 | 5526 | 8.6 | 0.0015 |
| | 3 | 381 | 138 | 102 | 2026 | 124 | 42 | 2813 | 4.0 | 0.0014 |

# Effects of Forest Spraying with DDT in New Brunswick on Food of Young Atlantic Salmon[1,2]

By Miles H. A. Keenleyside[3]

*Fisheries Research Board of Canada*
*Biological Station, St. Andrews, N.B.*

## ABSTRACT

Food in stomachs of young Atlantic salmon from the Northwest Miramichi River, N.B., changed following aerial DDT spraying of the surrounding watershed. Before spraying, young salmon typically ate immature aquatic insects, fry (underyearlings) concentrating on Diptera and small Ephemeroptera, and parr (overyearlings) on Diptera, Trichoptera, and all sizes of Ephemeroptera. Reduction of all aquatic insects by DDT was soon followed by resurgence of Chironomidae and other Diptera, and surviving fry fed heavily on these; parr fed on Diptera but also ate snails, worms, and fish, previously unimportant in their diet. Five years after the last spraying the pre-spray complexity of young salmon food was being approached. Trichoptera were slowest of the major fish-food types to reappear in stomachs.

In fry stomachs Ephemeroptera, and in parr stomachs Ephemeroptera and Trichoptera, were relatively more abundant than in the surrounding stream fauna. Greater availability of these insects and active selection by fish are discussed as possible causes of these relationships.

## INTRODUCTION

Young atlantic salmon in fresh water feed mainly on immature aquatic stages of insects, particularly Ephemeroptera, Trichoptera, and Diptera (White, 1936; Elson, MS, 1940; Carpenter, 1940; Allen, 1941; Hurley, MS, 1956). Many of these insects are killed when DDT insecticide is sprayed on forests and then enters the rivers in which they live (Ide, 1957). After repeated spraying some species of insects do not return to their pre-spray abundance for several years (Ide, 1967). Young salmon also are susceptible to poisoning by very low concentrations of DDT in water (Gagnon, 1958; Keenleyside, 1958), and Langford (1949) has shown that several species of freshwater fish are killed by ingesting DDT with their food. Repeated aerial application of DDT since 1952 to control an outbreak of spruce budworm in New Brunswick (Kerswill, 1967) thus affected resident young salmon directly through toxic action and indirectly by altering their food supply.

In the Miramichi River, N.B., most young salmon spend 3 years in fresh water before going to sea; a few spend 2 or 4 years (Blair, 1935).

The DDT insecticide was applied by low-flying airplanes at a rate of ½ lb DDT per ½ U.S. gal solution (DDT plus oil) per acre (Webb, 1959).

Hurley (MS, 1956) studied the effects of the 1954 DDT spraying on food of young Northwest Miramichi River salmon by comparing stomach contents

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[2] The fifth in a series of papers on work of the Fisheries Research Board of Canada on effects of forest insecticides on salmon and aquatic insects.

[3] Present address: Department of Zoology, University of Western Ontario, London, Ont.

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of fish caught in the same parts of the river in 1953 and 1955. He also compared the food of fish captured in 1955 with aquatic insects collected during the same year at locations inside and outside the 1954 spray zone. He found that changes in insect fauna reported by Ide (1957) were reflected in altered diets of the young salmon.

The present paper describes changes in the food of young salmon collected between 1953 and 1961 in the Northwest Miramichi River, in an area of New Brunswick sprayed with DDT several times during that period. For some years comparisons are made between food organisms found in fish stomachs and the aquatic insect fauna living in areas where the fish were collected.

Figure 1 shows the river and the stations where fish and insects were collected for this study. Parts of the river were within the spray zone in 1954, 1956, and 1957. In 1954 all the watershed from the headwaters to an area between stations 7 and 8 was sprayed. The 1956 spraying covered most of the watershed below station 3; fish-collecting stations downstream from station 3 were either within or immediately downstream from sprayed areas. In 1957 most of the watershed below station 4 was sprayed. From 1957 to 1962 no further spraying was done upstream from or near station 4.

## MATERIALS AND METHODS

Food of young salmon was studied by analyzing stomach contents of two series of fish. The first series was taken from collections made in the late summer of each year from 1953 to 1956, during annual fish population censuses on the Northwest Miramichi River (Elson, 1967). Among these were fish examined by Hurley (MS, 1956) with additional specimens to make up, wherever possible, a total of 10 fry and 10 parr from each of several locations each year. Fry used

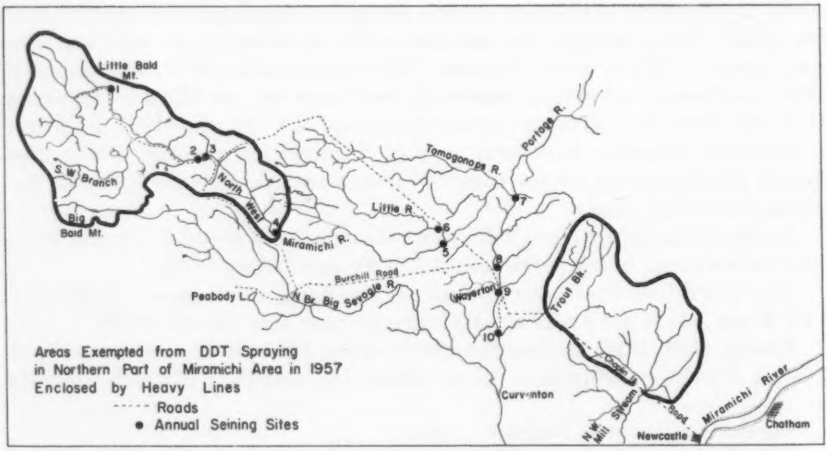
Little Bald Mt.
1
2
3
S. W. Branch
North West
4
Big Bald Mt.
Miramichi R.
Tomogonops R.
Portage R.
7
Little R.
6
5
8
9
Wayerton
10
Burchill Road
Peabody L.
N. Br. Big Sevogle R.
Trout Bk.
Chaplin Is.
Curventon
N. W. Mill Stream
Road
Newcastle
Miramichi River
Chatham
Areas Exempted from DDT Spraying in Northern Part of Miramichi Area in 1957 Enclosed by Heavy Lines
----- Roads
● Annual Seining Sites

FIG. 1. Northwest Miramichi River and its tributaries, showing locations of fish population census stations.

for stomach analysis were all underyearling fish and were relatively uniform in size. Parr were selected at random from samples of fish preserved during population census work on this river, and fish of various ages were thus used (Table IV gives age determinations of parr collected in 1959, 1960, and 1961).

The second series of fish was collected at 2-week intervals between late May and early September of 1959, 1960, and 1961 at stations 3 and 4. Each collection, whenever possible, consisted of 10 fry and 10 parr from each of the two stations. Fry were sometimes not available on the earliest collection dates because of their small size at that time. Fish in this series were caught near yard-square stream cages used for sampling emerging adult insects. Insect sampling at stations 3 and 4 was part of the long-term study of effects of DDT spraying on aquatic insects (Ide, 1967). It was thus possible to compare the food of these fish with the insect fauna of the same areas throughout the summer.

All fish were captured with electrofishing gear and a seine (Elson, 1962), and were preserved in 4% formalin. Some fish in the earlier series (1953–56) were stored in formalin for several years before examination. These fish were measured (fork length) before stomach analysis but because of dehydration during long storage they were not weighed. Fish collected in 1959, 1960, and 1961 were weighed and the total length recorded as soon as possible after capture, usually on the same day. Stomachs of all fish were removed and stored in 95% ethyl alcohol. The esophagus and mouth of each fish were examined before stomach removal and any food organisms lodged there were stored with the stomach. Only food in the fundus of the stomach was examined, since advanced digestion usually made food in the pyloric region unidentifiable. Stomach contents were classified to order (to family in Diptera) in the same manner as the collections by Ide (1967). Organisms were counted and the volume of the total contents was estimated by the liquid displacement method.

## RESULTS

### FOOD IN YEAR BEFORE SPRAYING

Fish caught in 1953 provide information on pre-spray feeding conditions in the river. Parr stomachs contained more and larger food organisms than fry (Table I); the greater mean volume for parr is associated with body size, usually greater in parr. Differences in composition of food taken by fry and parr also contributed to the differences in total numbers and volume. Chironomid larvae and pupae (among the smallest organisms eaten by young salmon) made up over 50% of fry food, but less than 25% of parr food (Fig. 2 and 3, upper charts). Ephemeroptera, Trichoptera, and Plecoptera, which are relatively large insects, formed higher proportions of the food of parr than of fry.

### FOOD IN YEAR OF AND IN YEARS AFTER SPRAYING

In 1954 no fry were found in the river during the annual population census, owing to high mortalities following DDT spraying in June of that year (Kerswill and Elson, 1955). Parr samples were collected at seven stations, however,

TABLE I. Food of salmon fry and parr collected in late summer, 1953–56, in Northwest Miramichi River. Fish collecting stations shown in Fig. 1.

| Year | Collecting stations | Stations sprayed | No. of fish | Mean length (*range*) | Mean number organisms per stomach | Mean volume food per stomach |
|---|---|---|---|---|---|---|
| **Fry** | | | | *cm* | | *ml* |
| 1953 | 1, 2, 3, 5, 8, 10 | None | 45 | 4.4<br>(3.6–5.1) | 14.0 | 0.018 |
| 1954 | 1, 2, 3, 4, 5, 6, 7 | All | 0 | – | – | – |
| 1955 | 1, 2, 3, 5, 9, 10 | None | 60 | 4.7<br>(3.6–5.7) | 34.9 | 0.019 |
| 1956 | 1, 2, 3 | None | 30 | 3.7<br>(3.1–4.4) | 11.5 | 0.028 |
| | 5, 9, 10 | All | 22 | 4.1<br>(2.9–4.8) | 13.9 | 0.025 |
| **Parr** | | | | | | |
| 1953 | 1, 2, 3, 5, 8, 10 | None | 59 | 9.0<br>(6.5–14.3) | 21.1 | 0.083 |
| 1954 | 1, 2, 3, 4, 5, 6, 7 | All | 69 | 9.8<br>(6.5–13.4) | 47.0 | 0.066 |
| 1955 | 1, 2, 3, 5, 9, 10 | None | 60 | 11.8<br>(7.2–16.8) | 36.4 | 0.282 |
| 1956 | 1, 2, 3 | None | 30 | 8.2<br>(6.8–13.2) | 32.4 | 0.048 |
| | 5, 9, 10 | All | 29 | 7.5<br>(6.1–13.2) | 13.3 | 0.063 |

apparently because they were less susceptible than fry to poisoning by DDT. The mean number of food items found in parr stomachs was more than double that found in 1953, but the mean volume of this food per stomach had declined (Table I). Parr stomachs in 1954 contained little other than Chironomidae and other Diptera (Fig. 3).

In 1955 the Northwest Miramichi watershed was not sprayed and both fry and parr were available for stomach analysis. Fry had many more food organisms per stomach than in the pre-spray year but the average volume of food per stomach was about the same (Table I). This was because they ate many more Chironomidae and other Diptera and fewer larger forms, such as Trichoptera, Ephemeroptera, and Plecoptera (Fig. 2). Parr in 1955 had fewer organisms per stomach than in 1954 but more than in 1953 (Table I); the mean volume of food was higher in 1955 than in any other year of this study (Tables I and IV). This volume increase in 1955 was partly because of an increase in the Miscellaneous category, made up of Gastropoda, Oligochaeta, and a few small fish (Cyprinidae), and partly because of the large average size of fish this year, smaller parr having been shown (Elson, 1967) to be more vulnerable than larger parr to DDT.

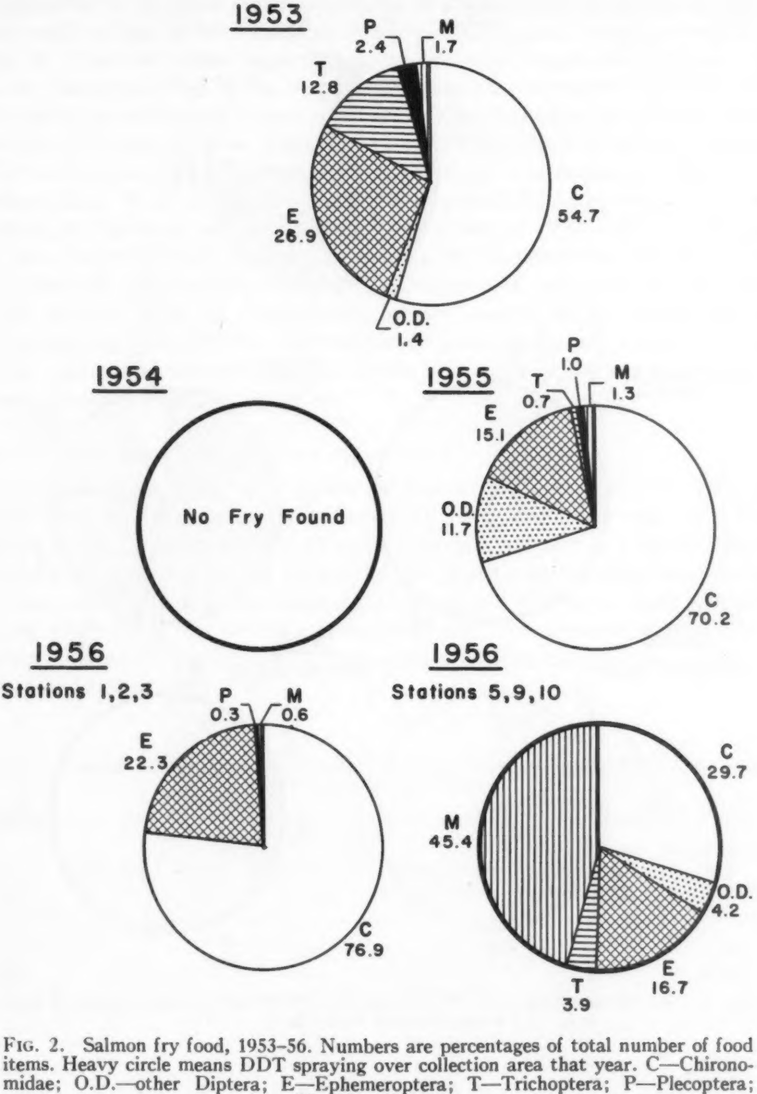

FIG. 2. Salmon fry food, 1953–56. Numbers are percentages of total number of food items. Heavy circle means DDT spraying over collection area that year. C—Chironomidae; O.D.—other Diptera; E—Ephemeroptera; T—Trichoptera; P—Plecoptera; M—Miscellaneous.

Data from fish collected in sprayed and unsprayed parts of the river in 1956 are presented separately in Table I and Fig. 2 and 3. In the unsprayed section (stations 1, 2, and 3) the mean number of organisms per fry stomach declined in 1956 to slightly below the pre-spray level, but the mean volume was

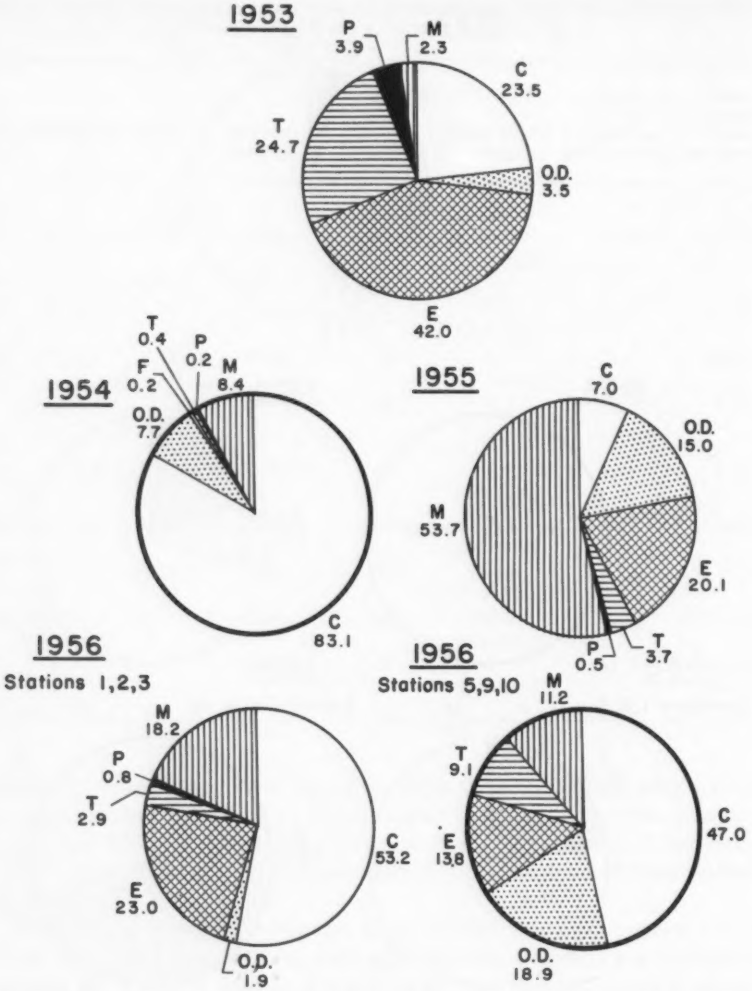

FIG. 3. Salmon parr food, 1953–56. Numbers are percentages of total number of food items. Symbols as in Fig. 2.

relatively high, even though fry stomachs contained virtually nothing but Chironomidae and Ephemeroptera. Parr from unsprayed stations in 1956 had more items per stomach than before spraying began, but the mean volume of this food was low, because of the preponderance of Chironomidae and Ephemeroptera. Snails and worms, which were so conspicuous in parr stomachs of 1955, were scarce in those of 1956.

Stomachs of fry from stations sprayed in 1956 contained about the same number and volume of food items as those from the upper, unsprayed sections (Table I). However, there were differences in food composition between fry from the two areas (Fig. 2). In the sprayed area Chironomidae and Ephemeroptera were proportionately scarcer and the Miscellaneous items much more abundant, the latter because of large numbers of Collembola in fish from station 10. The stomachs of parr from stations sprayed in 1956 contained fewer food organisms than those of parr from unsprayed areas; the mean volume of food per stomach, however, was greater among the former (Table III). This may have been caused by the smaller proportions of Chironomidae and Ephemeroptera and the greater proportion of Trichoptera in stomachs of parr from sprayed stations (Fig. 3). Trichoptera larvae, usually highly susceptible to DDT poisoning (Ide, 1957), were relatively more numerous in stomachs of both fry and parr from stations sprayed in 1956 than in fish from unsprayed stations the same year (Fig. 2 and 3).

## FOOD DURING INSECT RECOVERY FROM DDT

Information on the food of young salmon during 3–7 years after spraying was obtained at two stations (Tables II–IV, Fig. 4–6). Fry and parr were collected in the summers of 1959–61 in an area sprayed only in 1954 (station 3) and in another sprayed only in 1954 and 1956 (station 4). In 1956 the upstream spray boundary was between stations 3 and 4 and the latter may not have received much DDT; in 1957 the spray zone was downstream from station 4. Data on emergence of adult insects into stream cages were obtained at the two stations in 1959–61 (Fig. 4–6).

TABLE II. Food of salmon fry collected throughout the summers of 1959–61 at stations 3 and 4, Northwest Miramichi River.

| Year | Collecting station | No. of fish | Mean length (*range*) | Mean weight (*range*) | Mean number organisms per stomach | Mean volume food per stomach |
|---|---|---|---|---|---|---|
| | | | *cm* | *g* | | *ml* |
| 1959 | 3 | 54 | 4.6<br>(3.0–5.8) | 1.3<br>(0.5–2.1) | 9.2 | 0.009 |
| | 4 | 53 | 5.2<br>(3.6–7.0) | 1.8<br>(0.5–3.7) | 16.9 | 0.012 |
| 1960 | 3 | 70 | 4.0<br>(2.5–6.5) | 0.9<br>(0.2–2.2) | 16.0 | 0.027 |
| | 4 | 53 | 5.1<br>(2.3–7.4) | 1.7<br>(0.1–4.3) | 41.2 | 0.027 |
| 1961 | 3 | 60 | 3.5<br>(2.5–4.7) | 0.4<br>(0.01–1.0) | 10.2 | 0.010 |
| | 4 | 40 | 3.6<br>(2.2–5.2) | 0.7<br>(0.2–1.5) | 8.3 | 0.011 |

In the 3 years 1959–61, salmon fry collected at station 4 were slightly longer and heavier than those from station 3 (Table II). This may have been caused by slightly higher summer water temperatures prevailing at station 4, which is about 7 river miles downstream from station 3. Parr from station 4 were also larger than those from station 3, but this was at least partly because of the higher average age of parr collected at station 4 (Table III).

In 1959, salmon fry and parr collected at station 4 had a higher mean number and volume of food organisms per stomach than those from station 3 (Tables II and III). Stream cage collections at the two sites in 1959 (Fig. 4) showed that Chironomidae and Ephemeroptera were relatively more abundant in the insect fauna at station 3 than at station 4, while Trichoptera, Plecoptera, and other Diptera were relatively more numerous at station 4, although at both locations Chironomidae and other Diptera made up almost 75% of the insects collected.

The food of fry collected at the two stations in 1959 differed little in per cent representation of insect groups. Chironomidae and Ephemeroptera made up over 90% of fry food items at both stations (Fig. 4). Food of parr in 1959 reflected more closely than that of fry the differences in relative abundance of

TABLE III. Food of salmon parr collected throughout the summers of 1959–61 at stations 3 (sprayed in 1954) and 4 (sprayed in 1954 and 1956), Northwest Miramichi River.

| Year | Collecting station | No. of fish | Mean length (*range*) | Mean weight (*range*) | Age (*years*) | Per cent | Mean number organisms per stomach | Mean volume food per stomach |
|---|---|---|---|---|---|---|---|---|
| | | | *cm* | *g* | | | | *ml* |
| 1959 | 3 | 86 | 8.9 | 8.0 | 1 | 46.5 | 15.0 | 0.033 |
| | | | (5.3–12.2) | (2.4–17.1) | 2 | 44.2 | | |
| | | | | | 3 | 9.3 | | |
| | 4 | 75 | 10.4 | 11.9 | 1 | 16.0 | 31.5 | 0.058 |
| | | | (5.9–16.4) | (2.4–27.3) | 2 | 66.7 | | |
| | | | | | 3 | 16.0 | | |
| | | | | | 4 | 1.3 | | |
| 1960 | 3 | 89 | 8.7 | 7.9 | 1 | 73.0 | 37.3 | 0.048 |
| | | | (4.4–12.6) | (1.0–20.1) | 2 | 24.7 | | |
| | | | | | 3 | 2.2 | | |
| | 4 | 80 | 11.2 | 16.2 | 1 | 55.0 | 30.0 | 0.058 |
| | | | (8.0–19.0) | (5.8–36.1) | 2 | 38.8 | | |
| | | | | | 3 | 6.3 | | |
| 1961 | 3 | 70 | 8.8 | 7.3 | 1 | 41.4 | 21.9 | 0.029 |
| | | | (5.4–12.4) | (2.0–17.6) | 2 | 41.4 | | |
| | | | | | 3 | 17.1 | | |
| | 4 | 70 | 9.6 | 9.9 | 1 | 30.0 | 24.6 | 0.041 |
| | | | (6.8–13.7) | (2.6–27.5) | 2 | 54.3 | | |
| | | | | | 3 | 15.7 | | |

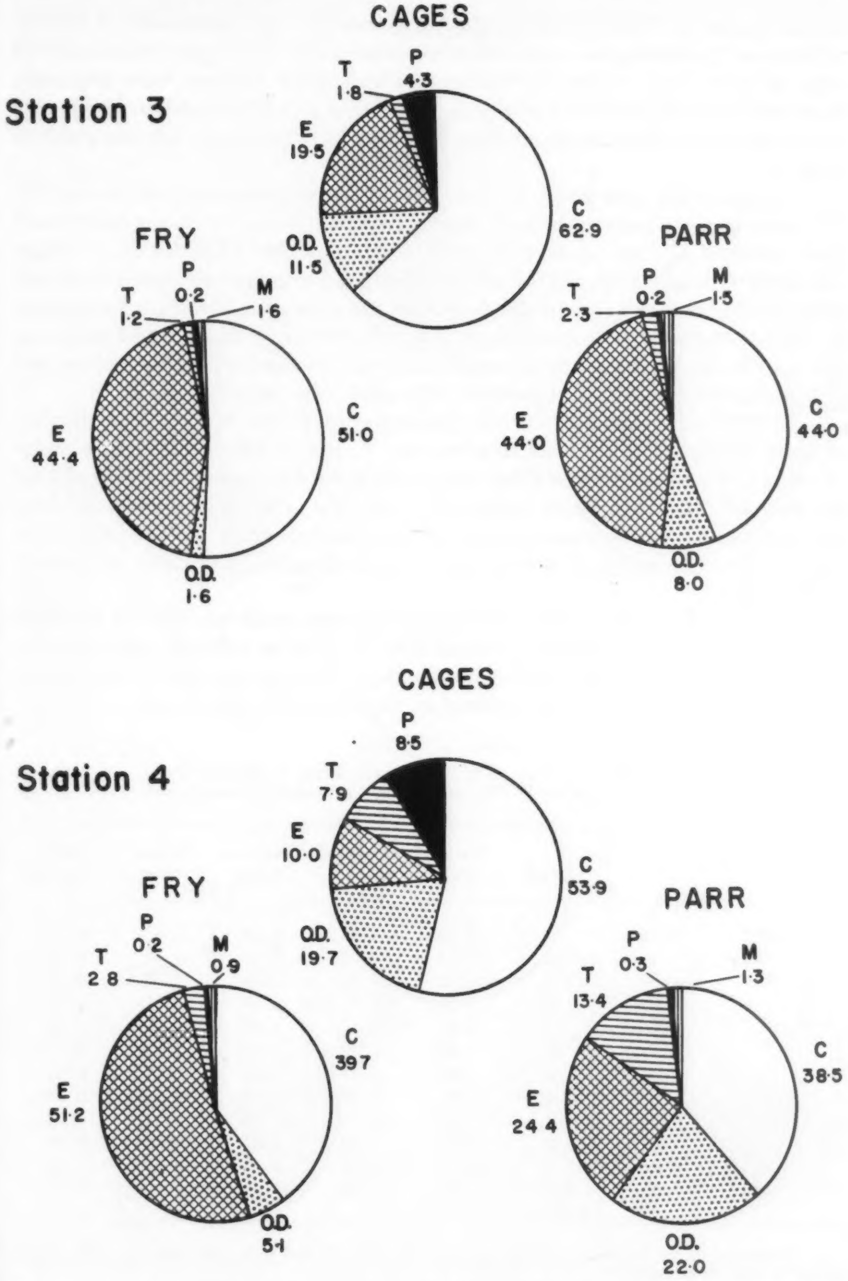

FIG. 4. Insect fauna and food of young salmon at stations 3 and 4 in 1959. Numbers are percentages of total number of organisms. Symbols as in Fig. 2.

insect groups at the two stations (as measured by cage collections). Chironomidae and Ephemeroptera were relatively more numerous in parr from station 3 than in parr from station 4; Trichoptera and other Diptera were relatively more numerous in parr from station 4. Plecoptera and Miscellaneous items were found in almost identical proportions in stomachs of parr from the two stations (Fig. 4).

Forage ratios (per cents, by numbers, of insect groups in food divided by per cents of same insect groups in cage collections) were calculated for fry and parr collected at both stations in 1959, 1960, and 1961 (Table IV). A forage ratio greater than one means the insects were found in greater relative abundance in fish stomachs than in cage collections from the same area. Among fry examined in 1959, forage ratios greater than one occurred only with Ephemeroptera. For parr in the same year forage ratios over one occurred with Trichoptera and Ephemeroptera at both stations and with other Diptera at station 4.

In 1960, stomachs of fry from station 4 contained a higher mean number of food organisms than those from station 3, but an identical mean volume (Table II). Parr from station 4 had fewer food items but a greater volume of food per stomach than those from station 3 (Table III). The differences in per cent representation of most insect groups between the two series of cage collections were small; Plecoptera, however, were relatively more abundant at station 3 (Fig. 5).

As in 1959, Chironomidae and Ephemeroptera made up most of the food of fry in 1960 at both collecting stations (Fig. 5). Parr in 1960 also concentrated largely on Ephemeroptera and Chironomidae. Plecoptera, which were abundant at station 3, were rarely utilized as food by either fry or parr.

TABLE IV. Forage ratios[a] for young salmon collected during 3 years at stations 3 (sprayed in 1954) and 4 (sprayed in 1954 and 1956), Northwest Miramichi River.

| Year | | Station | Plecoptera | Trichoptera | Ephemeroptera | Chironomidae | Other Diptera |
|---|---|---|---|---|---|---|---|
| 1959 | Fry | 3 | 0.05 | 0.67 | 2.28 | 0.81 | 0.14 |
| | | 4 | 0.02 | 0.35 | 5.12 | 0.74 | 0.26 |
| | Parr | 3 | 0.05 | 1.28 | 2.26 | 0.70 | 0.70 |
| | | 4 | 0.04 | 1.70 | 2.44 | 0.71 | 1.12 |
| 1960 | Fry | 3 | 0.05 | 2.47 | 1.68 | 1.02 | 0.26 |
| | | 4 | 0.40 | 0.28 | 2.78 | 0.36 | 0.21 |
| | Parr | 3 | 0.02 | 2.13 | 1.53 | 0.99 | 0.65 |
| | | 4 | 0.95 | 1.22 | 2.03 | 0.53 | 0.48 |
| 1961 | Fry | 3 | 0.03 | 2.73 | 0.70 | 1.33 | 0.21 |
| | | 4 | 0.04 | 0.58 | 0.92 | 0.95 | 0.90 |
| | Parr | 3 | 0.15 | 4.32 | 2.15 | 0.37 | 0.90 |
| | | 4 | 0.04 | 3.39 | 2.05 | 0.12 | 0.27 |

[a] Percentages, by numbers, of insect groups in food divided by percentages of same insect groups in cage collections.

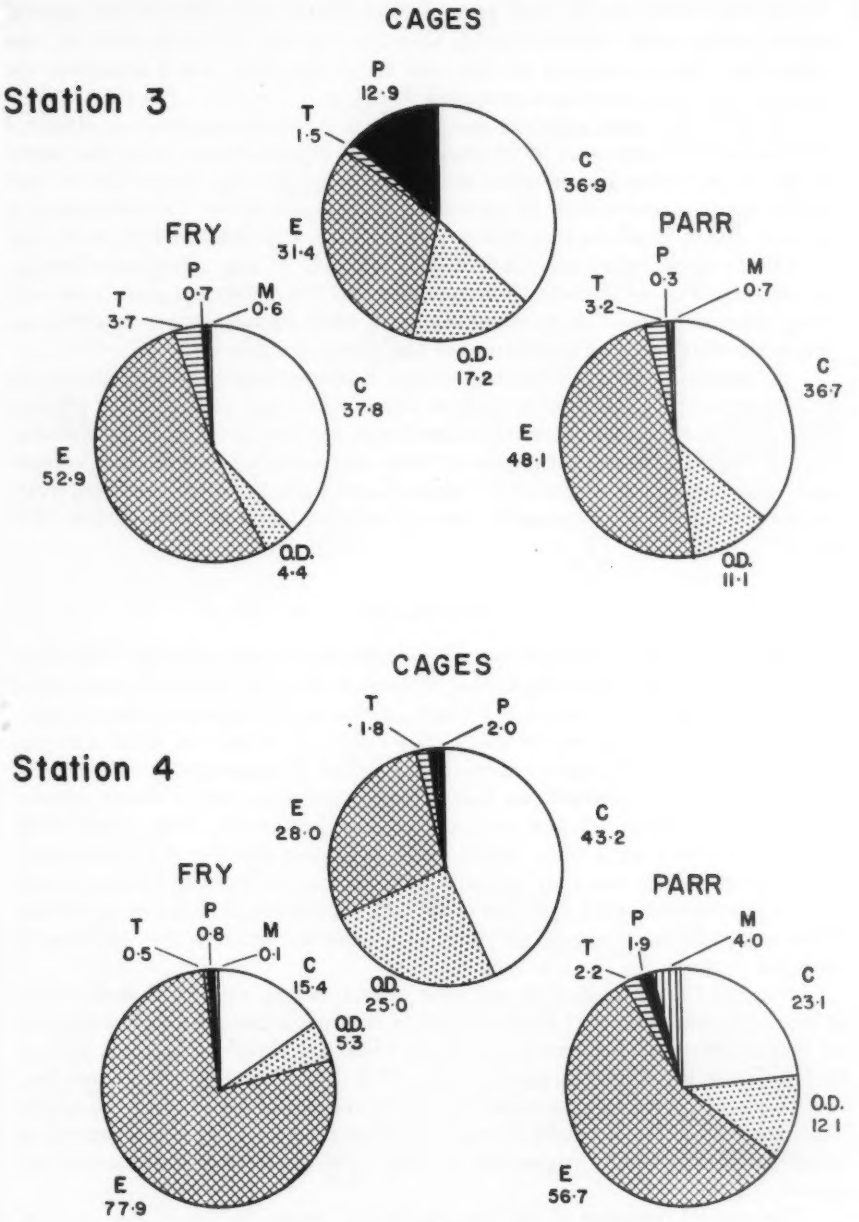

FIG. 5. Insect fauna and food of young salmon at stations 3 and 4 in 1960. Numbers are percentages of total number of organisms. Symbols as in Fig. 2.

Forage ratios for fry and parr in 1960 (Table IV) indicate that several insect groups were relatively more abundant in fish stomachs than in cage collections. Ephemeroptera for fry and Ephemeroptera and Trichoptera for parr are the most consistent examples.

In 1961 the mean numbers and volume of fry food organisms at station 3 had declined to about the 1959 level, while at station 4 they were the lowest in this 3-year intensive sampling program (Table II). The latter was at least partly due to unavailability of salmon fry at station 4 on two collection dates in August. Mean numbers and volume of parr food organisms in 1961 were likewise lower at both stations than in 1960 (Table III). Cage collections of emerging insects (Fig. 6) showed Chironomidae and other Diptera were relatively more abundant at station 3 than at station 4, while Ephemeroptera and Plecoptera were relatively more numerous in the fauna at station 4.

In general in 1961, Chironomidae and Ephemeroptera again made up the largest percentage by number of food items of fry and parr at both stations (Fig. 6). One exception occurred: at station 4, parr ate very few Chironomidae.

In 1961, fry forage ratios greater than one occurred only for Trichoptera and Chironomidae at station 3; Trichoptera and Ephemeroptera were relatively more numerous in parr stomachs than in the local fauna at both stations (Table IV).

## DISCUSSION

The present study has largely confirmed the conclusions of Hurley (MS, 1956) that DDT spraying of the Northwest Miramichi River in 1954 had pronounced effects on the insect fauna and as a result, on the food of resident young salmon. However, this more extensive study shows that the effects of DDT spraying on salmon food are somewhat more complex than he suggested.

The following observations support the suggestion that a direct relation exists between size of fish and size of their food (Lindstrom, 1955). While both fry and parr feed on a wide variety of insect types (Carpenter, 1940; Allen, 1941) Miramichi fry concentrated on smaller forms, particularly Chironomidae, other Diptera, and small Ephemeroptera. Parr generally had a diet consisting of large Ephemeroptera and Trichoptera, although on occasion they fed heavily on small Diptera.

Because Chironomidae recover soon after spraying with DDT and emerge in large numbers (Ide, 1957), fry which have not been killed are able to capitalize on their resurgence. Because larger insect types are slower to recover, parr at such times eat small insects, as they did in 1954 (Fig. 3), but even large numbers of Chironomidae made up a relatively small volume of food in parr stomachs that year (Table I). Generally, in such cases parr fed on a variety of organisms which are not normally important in their diet, such as aquatic snails and worms.

The second spraying of the Northwest Miramichi, in June 1956, appears to have had less drastic effects on the aquatic fauna than the first spraying in

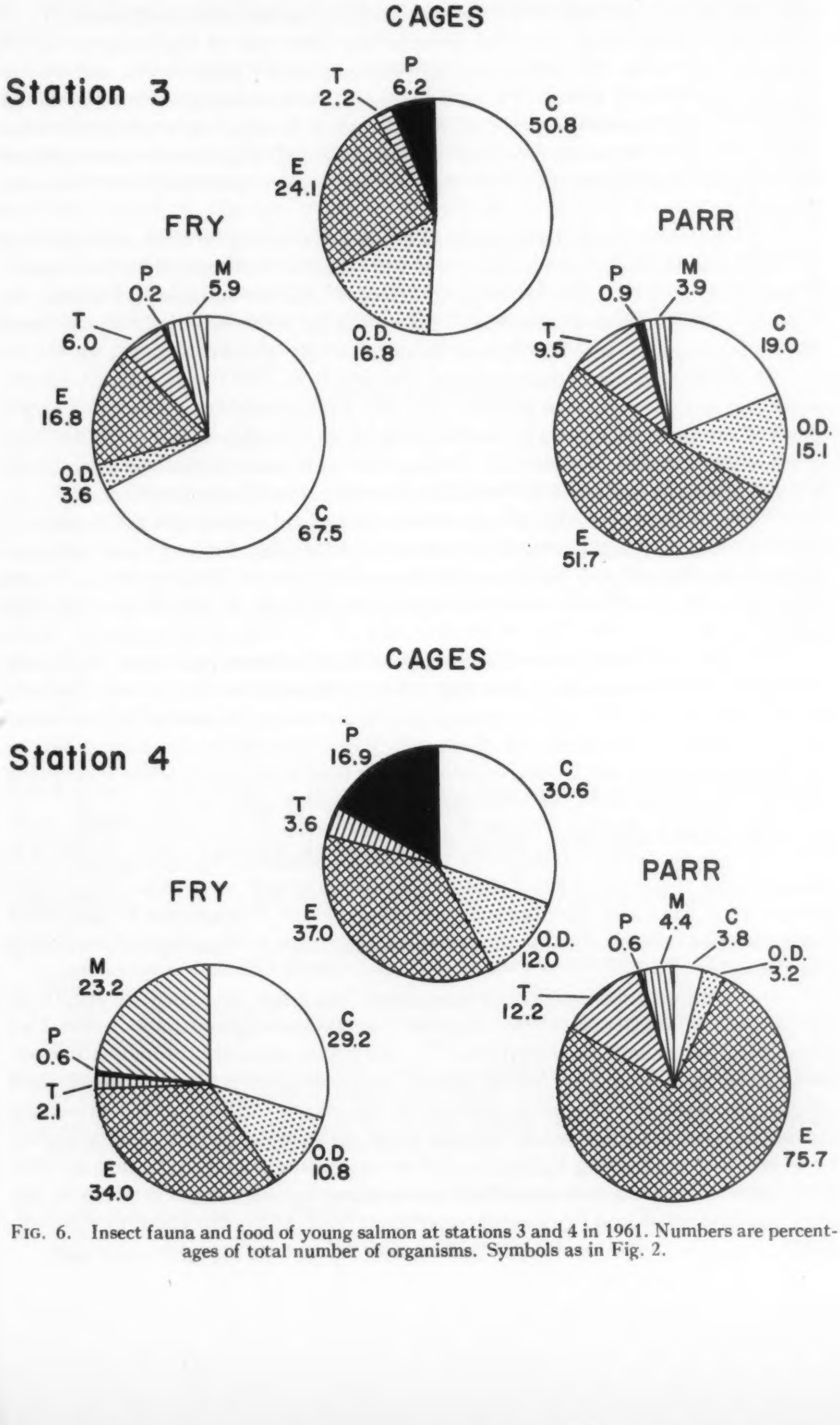

FIG. 6. Insect fauna and food of young salmon at stations 3 and 4 in 1961. Numbers are percentages of total number of organisms. Symbols as in Fig. 2.

1954. Moderate numbers of Trichoptera larvae, usually extremely sensitive to DDT (Ide, 1957), were found in 1956 in the stomachs of fry, more of which survived than after the 1954 spraying (Kerswill and Elson, 1955), and also in those of parr (Fig. 2 and 3). Probably the complete coverage of the Northwest Miramichi headwaters during the 1954 spraying resulted in a relatively large amount of DDT entering the river. In 1956 the upper parts were not sprayed and partial coverage of the lower stretches may have put less insecticide into the water.

The intensive sampling at stations 3 and 4, beginning in 1959, was expected to yield information about recovery of important fish food organisms. Collections of emerging insects indicated that, in terms of relative numbers of kinds, the aquatic fauna at these stations was diversified by 1959, especially at station 4 where Trichoptera and Plecoptera were relatively numerous (Fig. 4).

In 1960 continued recovery from the effects of DDT was evident among some but not all groups of insects (Fig. 5). Ephemeroptera made up a larger share of the cage collections at both sites than in 1959, but Trichoptera declined in relative abundance, especially at station 4. Plecoptera also were relatively less numerous at station 4 in 1960, but showed an increase at station 3.

Changes in utilization of aquatic insects as food by fry did not appear to follow any clear trends during 1959, 1960, and 1961 (Fig. 4–6). Fry concentrated mainly on Diptera and Ephemeroptera in all 3 years. They occasionally ate Trichoptera in small numbers but seldom ate Plecoptera, which are relatively large insects.

The only clear trends in the feeding habits of salmon parr over the 3-year recovery period were the increasing relative importance of Ephemeroptera, and the decreasing relative importance of Chironomidae. In general this followed similar trends in abundance of these two insect groups in the stream fauna. Trichoptera made a small contribution to parr food in all years, while Plecoptera were seldom eaten, even when numerous in the area (Fig. 6).

The forage ratio data (Table IV) show the types of insects most frequently eaten by young salmon, relative to the insect's abundance in the local stream fauna. With fry, the forage ratios for Ephemeroptera were most frequently greater than one; with parr both Ephemeroptera and Trichoptera forage ratios were always over one. Ratios for other insect groups were generally much less than one.

The food selection-availability problem has been discussed at length in the literature. Carpenter (1940) studied the food of salmon parr in the Dee River for 2 years and concluded that "... the fishes are indiscriminately carnivorous, and that their food varies strictly in accordance with the number and relative accessibility of species present in the rivers." Allen (1941) presented statistical evidence that young salmon show no selection when food is scarce, or when they are feeding lightly, but show increasingly more selection as they feed more heavily. Other workers have discussed predator–prey relations between salmonid fish and freshwater organisms (Neill, 1938; Morgan and Waddell, 1961).

The present study was not designed to investigate the availability-selection problem as applied to the feeding of young salmon. The following tentative conclusions are drawn, however, regarding vulnerability of potential food organisms to them: Ephemeroptera are highly vulnerable to predation by all sizes of young salmon, even when they form only a small proportion of the stream insect fauna; Trichoptera are also vulnerable, especially to larger young salmon; Plecoptera are seldom eaten, even when abundant; Chironomidae and other types of Diptera are eaten by young salmon of all sizes although, because these insects are very small, large numbers must be eaten to equal in volume a smaller number of larger insects. In the absence of immature aquatic insects young salmon can live on other organisms such as snails, worms, and small fish. Parr eat a greater variety of these than do fry, possibly because they can forage farther and more persistently than the smaller fry.

## ACKNOWLEDGMENTS

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# Effects on Aquatic Insects of Forest Spraying with Phosphamidon in New Brunswick[1,2]

By C. D. Grant[3]

*Fisheries Research Board of Canada*
*Biological Station, St. Andrews, N.B.*

## ABSTRACT

No harmful effects on aquatic insects were detected in two New Brunswick streams after forest spraying with phosphamidon (½–1 lb/acre) in 1962 and 1963. Comparison of one pre-spray and two post-spray series of bottom samples showed no reduction in number of orders (and families in the case of Diptera) and no abnormal decline in the number of individuals. In numbers and variety of insects emerging daily into stream cages and of insects drifting onto vertical screens in the streams, no changes were attributable to phosphamidon.

## INTRODUCTION

A SPRUCE BUDWORM epidemic in New Brunswick has led to extensive aerial spraying of forests with DDT (Kerswill, 1967). Ide (1967) has shown that such spraying seriously reduces aquatic insect populations. This report deals with a study of the effect of phosphamidon, one of the other insecticides tested to alleviate side effects (Kerswill, 1967).

## THE STREAMS

Locations of the study streams, Hughes Brook and McKenzie Brook, are shown in Fig. 1.

In 1962 part of Hughes Brook, a small, rapidly flowing stream that drops 350 ft in less than 3 miles and empties into the Taxis River, a tributary of the main Southwest Miramichi River, was sprayed with phosphamidon (1 lb/acre). In order to study effects of phosphamidon on aquatic insects within and downstream from the sprayed plot, two sampling stations were established: an "Upper Station" 200 yards inside the lower boundary of the spray plot and a "Lower Station" 2 miles downstream (Fig. 2). Both were visited daily from June 5 to July 1, 1962, for collections of invertebrate bottom fauna. To provide a comparison for the Hughes Brook bottom samples, collections were also taken from Longs Brook, an unsprayed stream 10 miles away on the other side of the Southwest Miramichi River.

In 1963 the McKenzie Brook system was sprayed with phosphamidon (½ lb/acre) for further studies. McKenzie Brook flows into the Nashwaak,

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[2] The sixth in a series of papers on work of the Fisheries Research Board of Canada on effects of forest insecticides on salmon and aquatic insects.

[3] Present address: Department of Zoology, University of Western Ontario, London, Ont.

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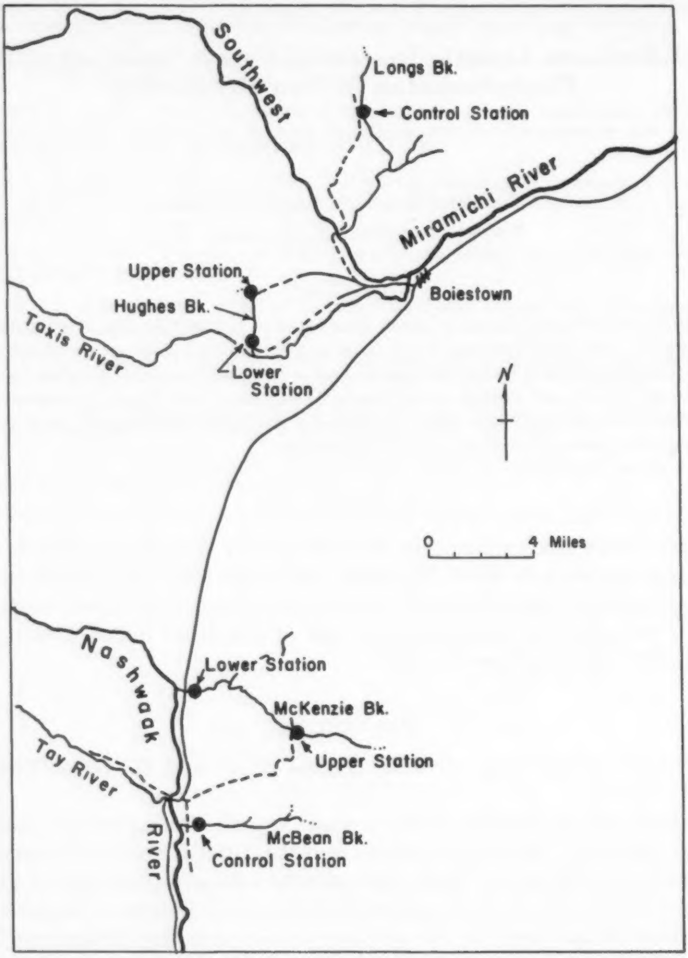
Southwest
Longs Bk.
Control Station
Miramichi River
Upper Station
Hughes Bk.
Boiestown
Taxis River
Lower Station
N
0
4 Miles
Nashwaak
Lower Station
McKenzie Bk.
Upper Station
Tay River
River
McBean Bk.
Control Station

FIG. 1. Streams in central New Brunswick used for studying effects of aerially applied phosphamidon (used as a control measure against spruce budworm) on aquatic insect fauna — Hughes Brook and Longs Brook in 1962, McKenzie Brook and McBean Brook in 1963. Thin-solid and broken lines, roads used.

a tributary of the Saint John River. Two stations were established on the brook: a "Lower Station" ½ mile from the mouth and an "Upper Station" 7 miles upstream from the mouth, on one of the two main branches (Fig. 3). In contrast to the block spraying of Hughes Brook in 1962, McKenzie Brook and its two branches for a total stream distance of about 15 miles were sprayed up and down the stream valley. Both stations were visited daily for collections

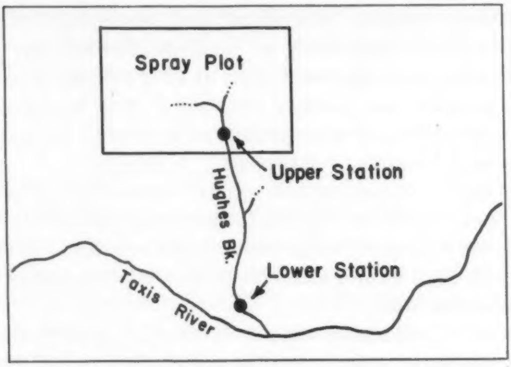
Spray Plot
Upper Station
Hughes Bk.
Lower Station
Taxis River

FIG. 2. Phosphamidon spray plot on Hughes Brook, June 9, 1962, and locations of the two stations used for studying effects of the spray on aquatic insects.

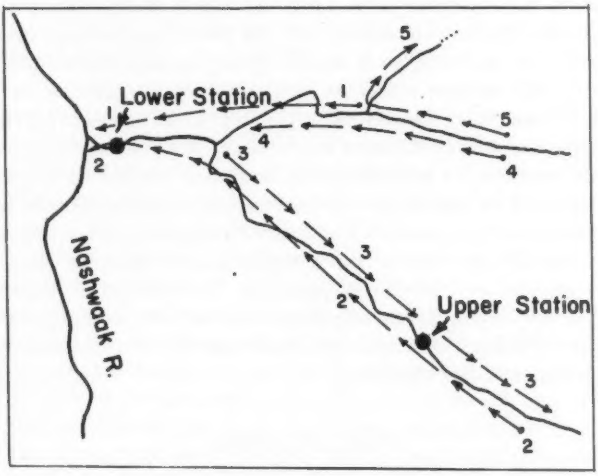
Lower Station
Upper Station
Nashwaak R.
1
2
3
4
5

FIG. 3. Flight paths used for aerial spraying of phosphamidon on McKenzie Brook, June 14, 1963, and locations of the two stations used for studying effects of the spray on aquatic insects.

from June 3 to July 1. McBean Brook (Fig. 1), entering the Nashwaak about 5 miles below McKenzie Brook, was not sprayed and was used for comparison for the bottom and emergence cage samples.

## METHODS

The following three methods used in earlier studies on DDT (Ide, 1967) were used to study effects of phosphamidon. Insects were classified to Order and in the case of Diptera to Family.

1. *Surber bottom samples.*—Pre-spray and post-spray collections of the bottom organisms were made with a Needham-Surber foot-square bottom sampler. Five samples were taken each time at each station. The stream bottom enclosed by the sampler was worked over for 5 min to dislodge organisms. Organisms and debris collected were preserved in alcohol for later classification and counting under a binocular microscope.

2. *Emergence cages.*—Emergence of aquatic insects was followed by means of Ide's (1940) cage traps (Fig. 4). The cages were cleaned daily, starting several days before spraying and continuing about 3 weeks after. Insects were picked off the inside of the cage with a swab dipped in alcohol, preserved in alcohol, and sorted and counted later.

3. *Drift screens.*—Foot-square copper screens (25 mesh to the inch) devised by Dr. F. P. Ide were set vertically on the stream bed to catch aquatic insects and other organisms drifting down (Fig. 5, *upper*). Daily collections from these screens were made from several days before to about 3 weeks after spraying. In collecting each sample, the accumulated debris and organisms were scraped from the screen with a table knife (Fig. 5, *lower*), preserved in alcohol, and later sorted under a binocular microscope for identification and counting. The samples taken before spraying were used to give a background number of insects that lodged on the screens either as they drifted downstream or after they climbed on the screen from the stream bottom. That some insect larvae normally moved up from the stream bottom to the drift screens was indicated by the fact that there was often a heavier concentration of insects on the downstream part of the screen. The post-spray screen collections contained the background number of insects plus any casualties rendered incapable of holding their places by the spray. Besides the daily screen samples, hourly samples using additional screens were collected on the day of spraying. This procedure was effective in detecting any immediate kill (Ide, unpublished data). Water levels were recorded for Hughes and McKenzie brooks to show whether sudden changes in level affected the number of insects taken.

## SPRAY HISTORIES

Before 1962, Hughes Brook had been sprayed three times with DDT: in 1957, 1960, and 1961. The Longs Brook control station had received DDT four times: in 1956, 1957, 1960 (when the upper boundary of the spray area probably included the station), and 1961. In 1962 the headwaters were again sprayed with DDT. Because of this 1962 spraying, which occurred late in June, only two series of bottom samples taken before the DDT was applied were used for comparison with the results from Hughes Brook. The Hughes Brook plot was sprayed between 6 and 7 AM on June 9, 1962.

Before the 1963 treatment with phosphamidon, McKenzie Brook had received DDT four times: in 1958, 1960, 1961, and in 1962 above the forks. McBean Brook had been sprayed in three previous years: 1958, 1960, and 1961. McKenzie Brook was sprayed with phosphamidon on the morning of June 14,

1963. The insecticide had been dyed blue and special dye-absorbing cards placed at the two stations showed that the stream was well sprayed. Foam that usually collected inside the cage at the Upper Station was tinged blue that morning.

## RESULTS AND CONCLUSIONS

The results from both years are discussed under the following headings.

1. *Surber bottom samples.*—The results of the three series of bottom samplings, a pre-spray series and two post-spray series, show no reduction in the number of orders and families considered (Tables I and II). In Hughes Brook a decrease in the numbers of mayfly nymphs and some other groups in the third series, taken in late June and on July 1, was probably the result of previous emergence of early-emerging species and partial drying up of the stream above the Upper Station. A further series of samples taken 13 months after spraying showed no decrease in the number of groups present, and Ephemeroptera, Chironomidae, and Trichoptera, the main fish food types (Keenleyside, 1967), showed an increase in the number of individuals. At McBean Brook the numbers in the samples increased during the test period and chironomid larvae were especially numerous, a characteristic of streams sprayed one or more times with DDT.

These results are in contrast to the observations following DDT spraying, after which many species were reduced to low numbers and recovery took years (Ide, 1967). Also, in reporting on Surber sampling of aquatic fauna during field testing of phosphamidon for forest insect control in Connecticut, Hitchcock (1965) concluded that it apparently presented limited danger to aquatic insects, as compared with the danger associated with forest spraying with DDT.

2. *Emergence cage samples.*—At the Upper Station on Hughes Brook there was a decrease in total number of insects emerging daily into cages (Fig. 6) as the stream dried up, but there was no reduction in the number of groups. At the Lower Station the emergence pattern fluctuated but there was also no reduction in the variety. At both stations on McKenzie Brook and at the control station on McBean Brook the daily collections showed a general increase in numbers as the season advanced. During the second week after spraying with phosphamidon, the emergence increased to very high numbers; the Lower Station was particularly rich in mayflies and stoneflies and the Upper Station produced large numbers of all types including miscellaneous Diptera. During the same post-spray period, chironomids emerged in great numbers from McBean Brook, thereby verifying the results obtained from the Surber samples.

This undisturbed emergence pattern was in contrast to the effect previously observed after DDT spraying of the Northwest Miramichi when emergence of insects was reduced to virtually nil for a period of from 3 to 6 weeks (Ide, 1967).

3. *Drift screen samples.*—The counts of descending insects collected at hourly intervals at the Upper Station on Hughes Brook and the Lower Station on McKenzie Brook (Fig. 7) show no increase after spraying. With DDT, on the

TABLE I. Total numbers of bottom organisms collected from five 1-ft$^2$ bottom samples in Hughes Brook before and after phosphamidon spraying on June 9, 1962, and in Longs Brook, a nearby unsprayed stream.

| Station | | Date | Ephemeroptera | Plecoptera | Trichoptera | Chironomidae | Simuliidae | Tipulidae Ceratopogonidae | Coleoptera | Odonata | Misc. larvae and pupae | Crustacea Arachnida | Annelida |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hughes Brook | Pre-spray | June 7, 1962 | 174 | 18 | 3 | 70 | 54 | 4 | 5 | 0 | 0 | 4 | 17 |
| *Upper Station* | Post-spray | June 19–20, 1962 | 191 | 45 | 14 | 230 | 314 | 4 | 4 | 1 | 0 | 6 | 22 |
| | Post-spray | June 29–30, July 1, 1962 | 72 | 86 | 3 | 416 | 0 | 3 | 3 | 4 | 7 | 6 | 59 |
| | 13 months post-spray | July 20, 1963 | 114 | 6 | 53 | 1154 | 23 | 11 | 2 | 10 | 6 | 1 | 60 |
| *Lower Station* | Pre-spray | June 7, 1962 | 154 | 40 | 10 | 368 | 63 | 0 | 2 | 0 | 0 | 1 | 40 |
| | Post-spray | June 20, 1962 | 168 | 51 | 17 | 283 | 643 | 1 | 3 | 1 | 2 | 0 | 0 |
| | Post-spray | June 29, 1962 | 51 | 56 | 10 | 453 | 734 | 1 | 4 | 5 | 6 | 8 | 81 |
| Longs Brook | | June 7–8, 1962 | 59 | 64 | 1 | 159 | 500 | 1 | 4 | 0 | 2 | 1 | 31 |
| *Control Station* | | June 20, 1962 | 94 | 75 | 6 | 341 | 850 | 5 | 0 | 0 | 6 | 0 | 78 |

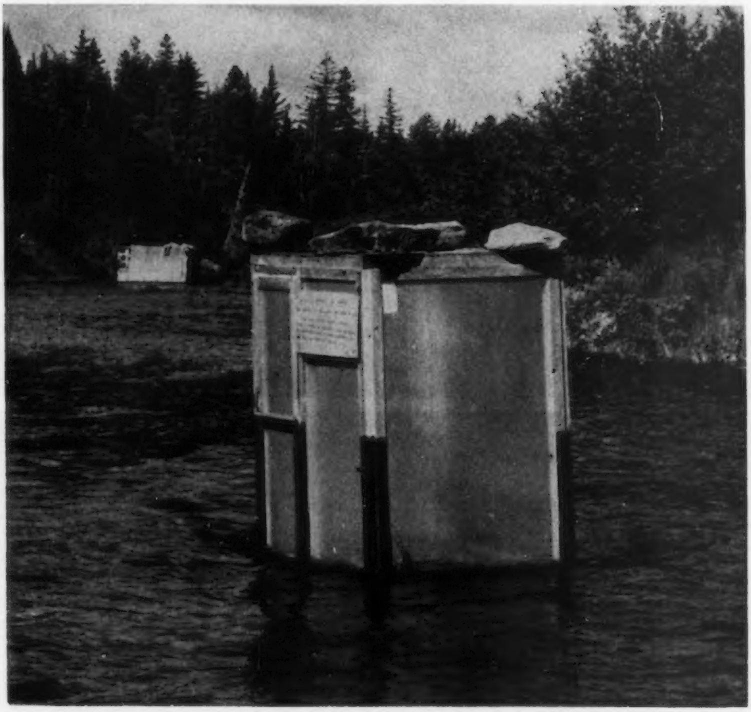

FIG. 4. Cage trap, for collecting emerging insects, at the Lower Station on McKenzie Brook, 1963.

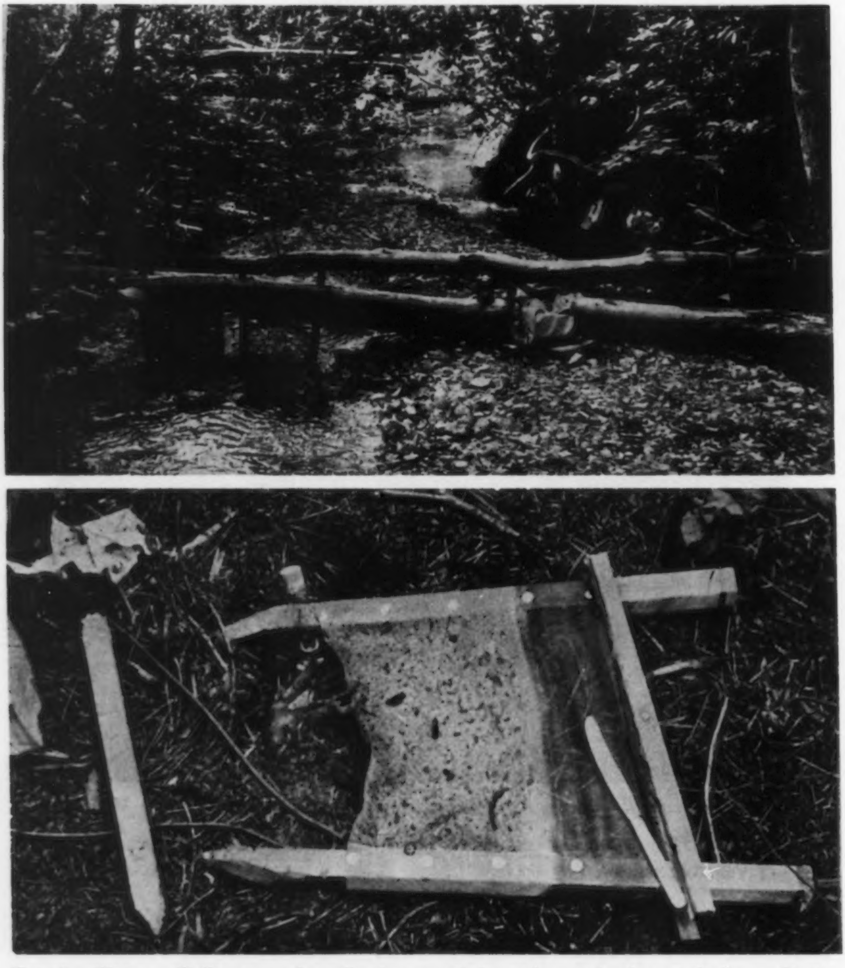

FIG. 5. *Upper* — Drift screens, for collecting aquatic insect larvae, in place at the Lower Station on Hughes Brook. *Lower* — A drift screen on the stream bank for cleaning; the screen was scraped with a table knife and the insects placed in a vial.

TABLE II. Total numbers of bottom organisms collected from five 1-ft² bottom samples in McKenzie Brook before and after phosphamidon spraying on June 14, 1963, and in McBean Brook, a nearby unsprayed stream.

| Station | | Date | Ephemeroptera | Plecoptera | Trichoptera | Odonata | Chironomidae | Simuliidae | Diptera larvae and pupae | Crustacea Arachnida | Mollusca | Annelida |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| McKenzie Brook | Pre-spray | June 11, 1963 | 261 | 25 | 10 | 1 | 426 | 18 | 7 | 15 | 1 | 11 |
| *Upper Station* | Post-spray | June 24, 1963 | 194 | 16 | 6 | 1 | 476 | 9 | 4 | 21 | 1 | 31 |
| | Post-spray | June 5–6, 1963 | 92 | 14 | 108 | 3 | 1532 | 63 | 11 | 23 | 5 | 27 |
| *Lower Station* | Pre-spray | June 11, 1963 | 324 | 32 | 17 | 0 | 197 | 0 | 13 | 6 | 12 | 22 |
| | Post-spray | June 24, 1963 | 423 | 6 | 39 | 1 | 174 | 1 | 9 | 11 | 19 | 7 |
| | Post-spray | July 5–6, 1963 | 167 | 3 | 31 | 2 | 307 | 10 | 10 | 13 | 12 | 0 |
| McBean Brook | | June 11, 1963 | 252 | 7 | 5 | 1 | 273 | 0 | 9 | 128 | 1 | 104 |
| *Control Station* | | June 24, 1963 | 192 | 12 | 7 | 1 | 1348 | 1 | 0 | 212 | 4 | 53 |
| | | July, 6, 1963 | 265 | 18 | 32 | 0 | 3534 | 5 | 12 | 154 | 1 | 157 |

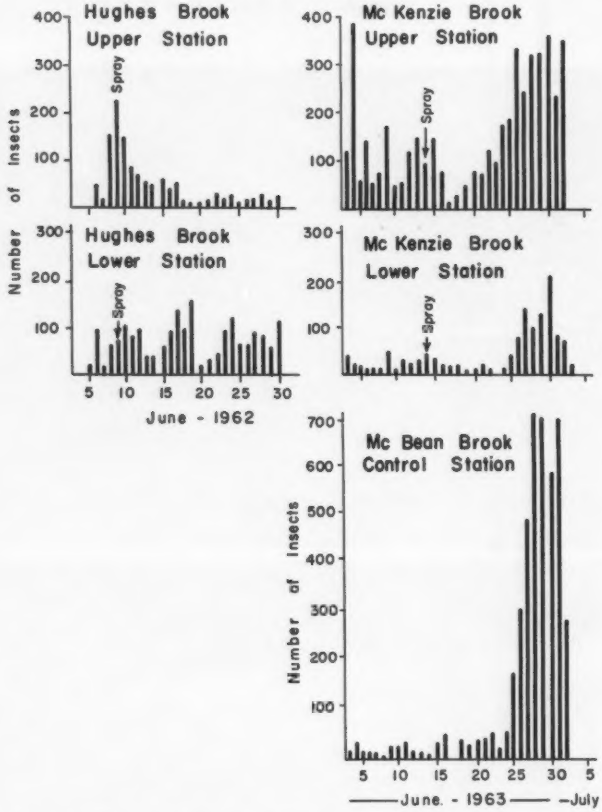

FIG. 6. Daily emergence of aquatic insects into 1-yard$^2$ cage at each station in Hughes Brook and McKenzie Brook, sprayed with phosphamidon, and in unsprayed McBean Brook (cages were not operated at Longs Brook).

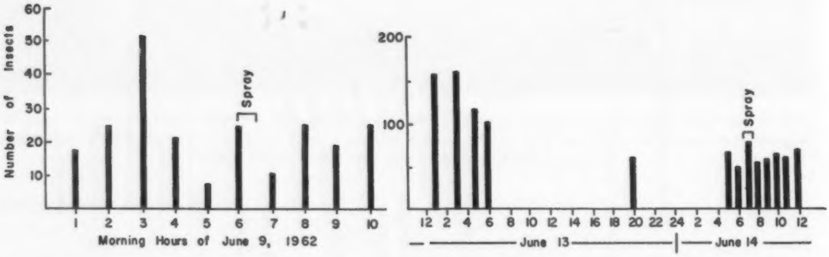

FIG. 7. Numbers per square foot per hour of aquatic insects collected from drift screens at the Upper Station on Hughes Brook on the day of spraying with phosphamidon in 1962 (*left*), and at the Lower Station on McKenzie Brook at selected hours on the day before and the day of spraying in 1963 (*right*).

other hand, it was found that within an hour or two of spraying the counts of insects on the screens increased drastically from background counts of less than 100 to 6000 or 8000 insects/hour (Ide, unpublished data).

The numbers of insects collected daily on the screens in Hughes and McKenzie brooks (Fig. 8 and 9) show no obvious changes due to spraying. There was

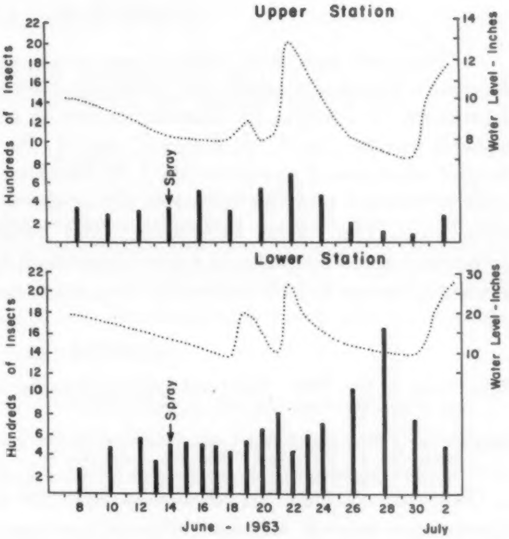

FIG. 8. Daily numbers per square foot of drift screen of aquatic insect larvae collected from Hughes Brook, before and after the brook was sprayed with phosphamidon on June 9, 1962.

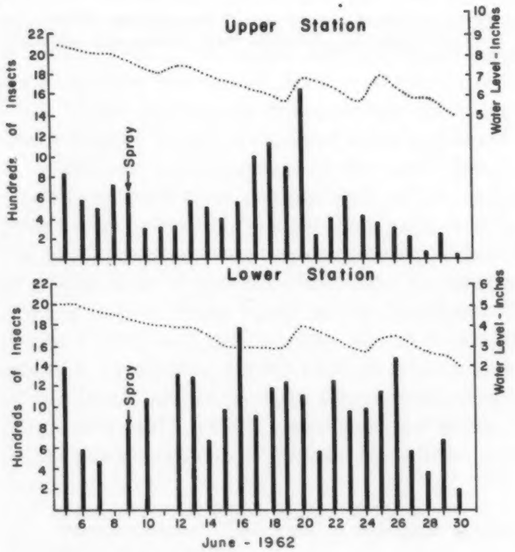

FIG. 9. Daily numbers per square foot of drift screen of aquatic insect larvae collected from McKenzie Brook, before and after the brook was sprayed with phosphamidon on June 14, 1963.

no consistent relationship between the water level and the number of insects taken on the screens. At the Lower Station on McKenzie Brook there was an increase in the number of insect larvae on the screen towards the end of June; during the same period lampreys seen spawning in the redds above this station likely stirred the bottom and dislodged insects.

## ACKNOWLEDGMENTS

This work was done while I was a summer student with the Fisheries Research Board of Canada. In particular I wish to thank Dr F. P. Ide of the University of Toronto for introducing me to and guiding me in the study of aquatic insects. Drs C. J. Kerswill and M. W. Smith provided direction and helpful criticisms of the work. Mr J. R. MacDonald of the Department of Fisheries of Canada kept me informed about the spraying program and provided other help. The Forest Entomology and Pathology Branch of the Canada Department of Forestry and Forest Protection Limited cooperated in planning the experiments and the latter did the spraying.

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# Echinoderms Collected from a Drifting Ice Island off the East Greenland Coast, with Comments on Their Distribution in Adjacent Waters[1]

By Rey Stendell[2]

*Department of Biological Sciences*
*University of Southern California, Los Angeles, California*

## ABSTRACT

A collection of 127 echinoderms was made from the ice island Arlis II as it drifted southward along the east coast of Greenland. Sixteen species are represented: 2 crinoids, 4 asteroids, 8 ophiuroids, 1 echinoid, and 1 holothurian. Distributions of the animals are discussed in relation to their occurrence in Greenland and adjacent waters. Of the species collected 14 are characteristic of the east Greenland marine fauna; two are characteristic of the basin floor of the northern North Atlantic and are not commonly found in east Greenland coastal waters, and two have been recorded from both regions.

## INTRODUCTION

Early in 1965, the ice island Arlis II (Arctic Research Laboratory Ice Station No. 2) left the central basin of the Arctic Ocean by way of the strait between Spitsbergen and Greenland and began to drift southward along the east coast of Greenland. By May of 1965, the island had drifted into the Denmark Strait between Iceland and Greenland and was evacuated on May 10 at 66°43′ N lat and 27°01′W long (Fig. 1). During this period, 72 bottom samples were taken of which 22 (31%) contained echinoderms. Altogether 127 specimens, representing 16 species, were taken. All five recent classes of the phylum are represented. Most of the species are characteristic members of the east Greenland fauna, which Heding (1935) considered to be composed of 48 species.

Previous investigations of the east Greenland marine fauna have been made from both land stations and surface ships. Collections along this coast have been hindered by the polar ice pack, which is present throughout most of the year. Ice conditions have prevented surface vessels from entering the area in winter months and have restricted penetration during the summer months. Studies such as those based on the Northeast Greenland Expedition by Mortensen (1910) and the Scoresby Sound Committee's 2nd East Greenland Expedition by Heding (1935) contain descriptions of the echinoderms collected in the fjords and immediate coastal waters of east Greenland. The works of Danielssen (1877, 1892), Danielssen and Koren (1882, 1884), and Grieg (1893) on the Norwegian North Atlantic Expedition, and Clark (1923), Heding (1942),

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[1] Received for publication August 15, 1966.

[2] Present address: Department of Biological Sciences, University of California at Santa Barbara, Goleta, California.

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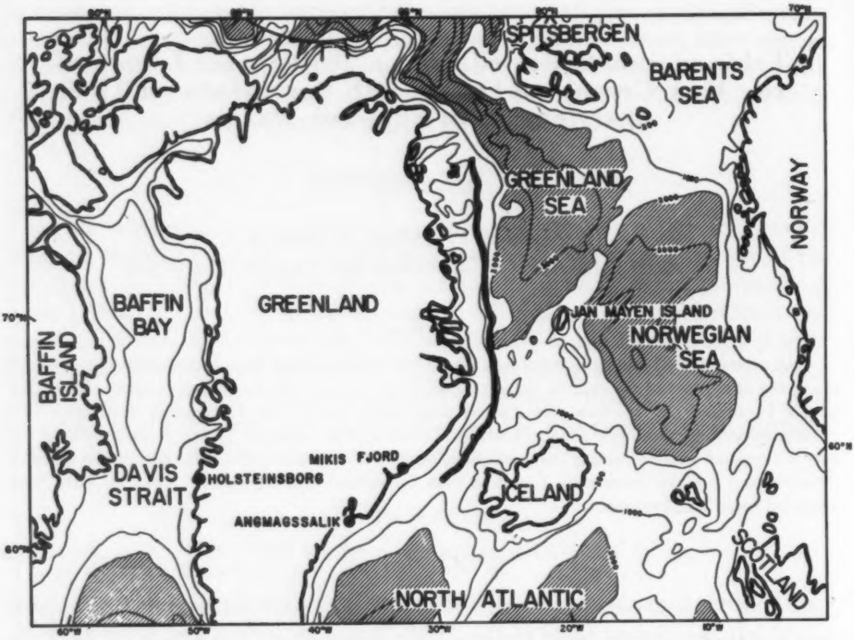
SPITSBERGEN
BARENTS SEA
GREENLAND SEA
NORWAY
GREENLAND
BAFFIN BAY
BAFFIN ISLAND
JAN MAYEN ISLAND
NORWEGIAN SEA
MIKIS FJORD
DAVIS STRAIT
HOLSTEINSBORG
ANGMAGSSALIK
ICELAND
SCOTLAND
NORTH ATLANTIC
70°N
60°N
60°N
60°W
50°W
40°W
30°W
20°W
10°W

FIG. 1. Northern North Atlantic region, showing Greenland and surrounding seas. Heavy line off the east coast of Greenland indicates the drift track of the ice island, Arlis II, during the period February through May 1965. Depth contours are in metres.

and Mortensen (1903, 1933) on the Danish Ingolf Expedition contain excellent accounts of the species collected in North Atlantic waters between Iceland, Jan Mayen Island, and Spitsbergen. Records of echinoderms collected in the area between the coastal waters and open North Atlantic are sparse, due to the great difficulties in collecting caused by the ice. As Arlis II was an integral part of the ice floe, it afforded excellent opportunity to collect from this region.

The purpose of this account is to document the echinoderms collected from Arlis II as it drifted southward between Greenland and the North Atlantic. The Arlis II list of species will add to the knowledge of the fauna of east Greenland and help elucidate changes in the fauna between the coast and deep sea.

## MATERIALS AND METHODS

As no standard bottom sampling equipment was available on Arlis II, two improvised samplers were utilized. A heavily weighted 1-gal bucket (3.8 litres) was employed for taking mud samples; 13 were taken, none of which contained echinoderms. Samples at the remaining 59 stations were taken with a $\frac{1}{4}$-inch (6.4-mm) screen dredge, constructed from the two halves of a wire minnow trap. The width of the scoop opening was 14 inches (35.6 cm). This device was of limited efficiency and the material collected cannot be considered quantitative. The drift of the ice island provided the movement necessary to operate both devices, as both functioned by dragging across and digging into the bottom to obtain a sample. It was hoped that the relatively large screen size would allow mud to wash through while the dredge was in operation. Apparently the consistency of the mud and rock bottom was such that the dredge filled up quickly with mud and operated more or less as a grab.

All echinoderms taken were fixed and preserved in 70% ethyl alcohol.

In October and November of 1964, three bottom samples were taken from Arlis II when it was northeast of Greenland in the vicinity of 84–85°N lat and 17–27°W long. Basically the same collecting equipment was employed as at the later stations, described above, but no echinoderms were collected.

In the following account of the echinoderms collected the number in parentheses after each station number indicates the number of specimens taken at that station. Location and water depth of the stations are given in the station list at the end of this account.

## Class CRINOIDEA
### Family BATHYCRINIDAE

*ILYCRINUS CARPENTERI* (Danielsen and Koren)

Records indicate that this arctic species is found in the deep regions of the northern North Atlantic as far south as 63°N (Clark, 1923; Danielssen, 1877). It is a deep-water species and has not previously been recorded from any waters adjacent to Greenland.

*Arlis II collections*: 2 specimens, 1615 and 1635 m. Stations 297(1), 319(1). Stalk lengths were 37 and 110 mm.

### Family ANTEDONIDAE

*POLIOMETRA PROLIXA* (Sladen)

This is an arctic species, found in both east and west Greenland coastal waters as well as the cold area of the North Atlantic (Clark, 1923; Danielssen, 1877; Heding, 1935; Mortensen, 1904, 1910). It has also been recorded from Spitsbergen and the Kara Sea, but records of its occurrence in Davis Strait or Baffin Bay are lacking (Mortensen, 1933).

*Arlis II collections*: 14 specimens, 205–355 m. Stations 173(4), 180(5), 196(3), 238(1), 332(1).

## Class ASTEROIDEA
### Family BENTHOPECTINIDAE

*PONTASTER TENUISPINUS* Duben and Koren

This widely distributed species is found in both arctic and northern Atlantic waters. Its range extends across the Canadian Arctic into the Kara and Siberian seas, southward into the North Atlantic as far as 42°N lat on the American side (Mortensen, 1927). Records show its being taken on the east coast of Greenland and at Spitsbergen, but not in the deep regions of the Greenland and

Norwegian seas (Mortensen, 1904, 1910). West of Greenland it is represented in the coastal regions as well as in Baffin Bay (Mortensen, 1932).

*Arlis II collections*: 2 specimens, 205–375 m. Stations 139(1), 150(1).

Both individuals are small, having radii of 8 and 9 mm. Specimens of such small size appear to be taken rarely, although Mortensen (1910) records an individual with a 6-mm radius collected from northeast Greenland.

### Family PTERASTERIDAE

*HYMENASTER PELLUCIDUS* Wyv. Thompson

East of Greenland, this predominantly cold-water form is found along the coast as well as in the deep regions of the Greenland and Norwegian seas to Spitsbergen and in the Siberian Sea (Danielssen and Koren, 1884; Mortensen, 1904). It has been recorded from Baffin Bay and northwest Greenland but not from the vicinity of the Davis Strait (Mortensen, 1932).

*Arlis II collections*: 1 specimen, 205 m. Station 173(1).

### Family KORETHRASTERIDAE

*KORETHRASTER HISPIDUS* Wyv. Thompson

This species has been recorded from the Faroe Channel and northern North Atlantic to Spitsbergen and the Kara Sea as well as along the east coast of Greenland (Danielssen and Koren, 1884; Heding, 1935). There are no records of this species having been collected in any west Greenland waters.

*Arlis II collections*: 1 specimen, 435 m. Station 210(1).

### Family ASTERIIDAE

*ICASTERIAS PANOPLA* (Stuxberg)

*Icasterias panopla* is an arctic species found on both the east and west coasts of Greenland and is believed to have a continuous distribution around the north coast (Mortensen, 1904, 1910). It apparently does not occur in the deep regions of the Greenland and Norwegian seas, but has been recorded at Spitsbergen and in Barents and Kara seas (Danielssen and Koren, 1884).

*Arlis II collections*: 1 specimen, 365 m. Station 88(1).

### Class OPHIUROIDEA
### Family GORGONOCEPHALIDAE

*GORGONOCEPHALUS EUCNEMIS* (Muller and Troschel)

This widely distributed arctic species has been collected from the east coast of Greenland in water from 50 to 500 m (Mortensen, 1904, 1910). It has not been recorded from Denmark Strait or along the coast south of Scoresby Sound. In west Greenland it has been recorded from the coastal areas, but not from Baffin Bay or the Davis Strait (Mortensen, 1932; Heding, 1936). It has not been recorded from the deep regions of the Norwegian Sea but has been collected from waters east of Iceland, at Spitsbergen, along the coast of Scandinavia as well as in the Greenland, Siberian, and Kara seas (Greig, 1893). It is believed to be a circumpolar species.

*Arlis II collections*: 1 specimen, 275 m. Station 44(1).

### Family OPHIOMYXIDAE

OPHIOSCOLEX GLACIALIS Muller and Troschel

This species has a fairly wide distribution in both warm and cold areas of the northern Atlantic, although on the European side it is found no farther south than the Faroe Channel (Mortensen, 1933). In the western Atlantic it appears to be distributed as far south as the West Indies (Mortensen, 1933). In the North Atlantic it has been recorded from waters around Iceland, Jan Mayen Island, and Spitsbergen (Greig, 1893). It is well represented in the collections from the East Greenland coast, having been found in the fjords as well as the water off the coast (Mortensen, 1904, 1910; Heding, 1935). It has been found on the west coast of Greenland, but appears to be not very widespread in this area (Mortensen, 1932).

*Arlis II collections*: 26 specimens, 205–1230 m. Stations 102(1), 132(1), 166(16), 173(3), 390(5).

Disk diameters range from 3 to 25 mm, with six individuals having disks greater than 20 mm and 15 less than 10 mm. All five individuals from Station 390 have disks between 23 and 25 mm.

### Family OPHIACANTHIDAE

OPHIACANTHA BIDENTATA (Retzius)

A circumpolar species, *Ophiacantha bidentata* has a wide distribution in warm Atlantic as well as cold arctic waters. It has been recorded from both the east and west coasts of Greenland, but not from the deep regions of the Norwegian Sea (Mortensen, 1932). It is well represented in the collections from the fjords and coastal waters of East Greenland (Mortensen, 1904, 1910; Heding, 1935). In the northern Atlantic it is recorded from the Faroe Channel, Denmark Strait, Jan Mayen Island, Spitsbergen, and the coast of Scandinavia (Greig, 1893; Mortensen, 1933).

*Arlis II collections*: 42 specimens, 205–435 m. Stations 111(1), 139(1), 150(5), 166(24), 173(1), 180(1), 210(5), 230(1), 332(3).

There is considerable variation in the specimens collected, especially in the shape of the dorsal arm plates. In most of the specimens the plates have the characteristic rhomboid shape and are separate from each other. A few individuals have plates with the distal edge rounded and in contact with the proximal edge of the adjacent plate, as in the variety *fraterna*, described by Mortensen (1933). The spinules of the disk have a characteristic stumpy appearance but there is some variation in the amount of disk covered by these spinules. Some disks are completely covered while others have the distal portions of the radial shields naked. Shapes of the disks vary from round to pentagonal.

One specimen from Station 166 possesses six arms, an anomaly in this species. Six mouth plates are present, all of which are equally developed. Mortensen (1933) mentions a seven-armed specimen, taken from the Kara Sea; only five normally developed jaws were present, a sixth being rudimentary.

### Family OPHIACTIDAE

#### *OPHIOPUS ARCTICUS* Ljungman

This predominantly cold-water form is known mainly from the Norwegian Sea but ranges into the Greenland Sea to Spitsbergen and down along the coast of Greenland to Iceland and into the Faroe Channel (Greig, 1893; Mortensen, 1933). Records show it from both the fjords and open coastal waters of the east and west coasts of Greenland (Mortensen, 1904, 1910, 1932).

*Arlis II collections*: 8 specimens 205–435 m. Stations 159(1), 166(1), 173(2), 210(3), 332(1).

Disk diameters of this small species range from 2 to 4 mm. Maximum disk size as given by Mortensen (1933) is 6 mm.

#### *OPHIOPHOLIS ACULEATA* (Linnaeus)

This widely distributed species is found in many locations in arctic and Atlantic waters; it is apparently a circumpolar form. In the North Atlantic it is found along the coast of Scandinavia, at Spitsbergen, around Iceland, and around Jan Mayen Island (Greig, 1893). Records of its occurence along the east coast of Greenland are almost lacking; Mortensen (1904) reports it to have been collected from 260 m at Angmasalik. West Greenland records are more abundant, the species having been recorded from Davis Strait as well as the fjords and offshore waters (Heding, 1936; Mortensen, 1932).

*Arlis II collections*: 1 specimen, 205 m. Station 173(1).

### Family OPHIOLEPIDAE

#### *OPHIURA SARSI* Lütken

A circumpolar species, *Ophiura sarsi* has a wide distribution in the North Atlantic. It apparently does not occur in the Norwegian Sea, but is recorded from the Faroe Channel, waters north of Iceland, Jan Mayen Island, Spitsbergen, and the Kara Sea as far as Novaya Zemlya (Greig, 1893; Mortensen, 1933). It has been recorded from Davis Strait and the west coast of Greenland (Heding, 1936; Mortensen, 1932). Records of its occurrence in East Greenland waters are sparse and it is absent from the northeast Greenland collection (Mortensen, 1910).

*Arlis II collections*: 1 specimen, 205 m; 22-mm disk diameter. Station 173(1).

#### *OPHIOCTEN SERICEUM* (Forbes)

*Ophiocten sericeum* is another ophiuroid with a wide distribution in the arctic and North Atlantic. Chiefly a cold-water species, it appears in almost all collections made along the east and west coasts of Greenland, in the fjords as well as off the coast. It has not been found in the deep regions of the Norwegian or Greenland seas, but has been recorded from waters off Iceland, Jan Mayen Island, Spitsbergen, and the coast of Scandinavia (Greig, 1893; Mortensen, 1933).

*Arlis II collections*: 11 specimens, 205–270 m. Stations 131(1), 150(5), 173(1), 180(3), 222(1). Disk diameters ranged from 4 to 12 mm.

### *OPHIOPLEURA BOREALIS* Danielssen and Koren

This arctic species does not occur south of the ridge separating the cold Norwegian Sea from the warm North Atlantic. It is a species characteristic of the cold area of the Norwegian Sea, having been recorded from Jan Mayen Island, Spitsbergen, the coast of Scandinavia, and on into the Barents and Kara seas (Greig, 1893; Mortensen, 1933). It is not known to be circumpolar. It has been recorded from the East Greenland coast in latitudes north of Iceland, but not from the Denmark Strait or latitudes south (Mortensen, 1904, 1910). Its distribution in West Greenland is limited, specimens having been found in Baffin Bay but not in the Davis Strait (Mortensen, 1932).

*Arlis II collections*: 3 specimens, 205–375 m. Stations 19(1), 139(1), 150(1). Disk diameters range from 20 to 23 mm.

## Class ECHINOIDEA
## Family STRONGYLOCENTROTIDAE

### *STRONGYLOCENTROTUS DROEBACHIENSIS* (O. Fr. Muller)

A widely distributed species in arctic and subarctic waters, *Strongylocentrotus droebachiensis* is represented in all collections from Greenland waters. It is the only echinoid recorded from the east coast of Greenland although several other species are found on the west coast. A circumpolar species, it has been recorded from Spitsbergen, the Siberian and Kara seas, Bering Strait, and the North Pacific (Danielssen, 1892; Mortensen, 1903). Records from the west coast of Greenland show it to occur along that coast but not from Davis Strait or Baffin Bay (Heding, 1936; Mortensen, 1932).

*Arlis II collections*: 13 specimens plus test fragments, 205–435 m. Stations 131(1), 166(12); test fragments from stations 173 and 210. Disk diameters vary from 9 to 23 mm with the majority in the 12–16 mm range.

## Class HOLOTHURIOIDEA
## Family ELPIDIIDAE

### *ELPIDIA GLACIALIS* Theel

This predominantly deep-water species is characteristic of the deep regions of the northern North Atlantic, but has also been found to occur in Baffin Bay west of Greenland (Mortensen, 1932). Records show it to have been taken from several stations northeast of Iceland and farther north and east into the Norwegian Sea (Danielssen and Koren, 1882; Heding, 1942). Collections along the east coast of Greenland have not revealed this species, although it is believed to have a continuous distribution around Greenland. It has been recorded from water as shallow as 70 m in the Kara Sea (Theel, 1876). Recent records (Hansen, 1956) from deep trenches in the Pacific and Indian oceans indicate that this species may be cosmopolitan.

*Arlis II collections*: 1 specimen, 1635 m. Station 319(1).

Hansen (1956) recognizes five geographical subspecies of *E. glacialis* by the number, size, and position of the dorsal papillae and, to a lesser degree, by the shape of the spicules. Northern forms may be distinguished from all other forms by the dorsal papillae being arranged in separate anterior and posterior groups; together they usually have less than four pairs of papillae. The form from the Norwegian Sea usually has four pairs, three anterior and one posterior; often extra papillae are present, but still in the anterior or posterior group. The same is the case with specimens from the deep regions (1800 m) of Baffin Bay, but specimens from shallower water (ca. 700 m) have five pairs of papillae, four anterior and one posterior. A considerable number (26%) of those collected by the Ingolf Expedition in the North Atlantic have only two pairs of anterior dorsal papillae.

The specimen in the Arlis II collections is small, with body length of only 8 mm. It possesses four pairs of locomotory podia and three pairs of dorsal papillae, two anterior and one posterior. The second anterior pair is considerably smaller than the first and is located almost directly over the first pair of podia.

## ZOOGEOGRAPHICAL CONSIDERATIONS

The North Atlantic region surrounding Greenland is subdivided into several deep-sea basins (Fig. 1). The Wyville Thompson Ridge, a shallow region stretching from north Scotland to Iceland, separates the boreal region of the North Atlantic from the cold arctic seas. The area north of the ridge comprises the Norwegian Sea, the major sea between Greenland and Scandinavia, and the Greenland Sea, between Spitsbergen and the northern part of East Greenland. The ridge continues across the Denmark Strait from northwest Iceland to Mikis Fjord in Greenland. West of Greenland, in Davis Strait, a continuation of the ridge completes the separation of cold arctic from warmer Atlantic water, by separating the deep basin of Baffin Bay from the Labrador Sea.

Many species characteristic of cold arctic regions are not restricted to areas north of the ridge. Nine species in the Arlis II collection are recorded from European boreal waters: *Pontaster tenuispinus*, *Gorgonocephalus eucnemis*, *Ophioscolex glacialis*, *Ophiacantha bidentata*, *Ophiopus arcticus*, *Ophiopholis aculeata*, *Ophiura sarsi*, *Ophiocten sericeum*, and *Strongylocentrotus droebachiensis*. The Greenland coastal fauna south of the ridge, i.e. south of Hosteinsborg and Mikis Fjord, is influenced by boreal waters and has a fauna separate from that north of the ridge. *Poliometra prolixa*, an arctic species not commonly found in boreal waters, has been recorded from the southeast coast.

Considerable confusion has arisen in attempts to define the bathymetrical zones off the east coast of Greenland. Mortensen (1910) described three zones in this area: littoral (0–200 m); archibenthal (200–1000 m); and abyssal (greater than 1000 m). He does not distinguish between the archibenthal and abyssal zones north of the Denmark Strait because of the uniformity of the fauna below the littoral zone. Mortensen (1933) considers only littoral and abyssal zones with the limit between them not clearly defined, but somewhere between 200 and 500 m. Each zone has an assemblage of species peculiar to it and not found in the adjoining zone.

Menzies (1962) states that by designating the start of the abyss at 1000 m, the entire arctic basin would be a single unit and suggests that 2000 m has more topographic reality as it would separate the major basins of arctic waters. He gives the following faunistic separation: shelf (0–200 m); slope (200 to 2000–3000 m); and basin floor (greater than 2000–3000 m). Most of the species collected from Arlis II have a considerable range of depth, but if we accept Menzies' definition, almost all can be considered characteristic of the slope. The shelf along the greater part of the East Greenland coast is narrow and apparently no echinoderm species are confined to it. Most Arlis II species are characteristic of the slope and extend up onto the shelf.

Previous East Greenland collections were generally made in depths of 500 m or less (i.e. shelf and upper slope) and the species represented in them have comprised the known East Greenland echinoderm fauna. All but two species in the Arlis II collection can be considered typical of this fauna. The two exceptions, *Ilycrinus carpenteri* and *Elpidia glacialis*, are typically basin-floor species, which do extend up the slope (as defined by Menzies). *Elpidia glacialis* is occasionally found on the shelf in high arctic regions, but most finds have been in considerable depths. It has also been recorded from Baffin Bay. *I. carpenteri* has not been found in Baffin Bay.

Two species, *Ophiopleura borealis* and *Hymenaster pellucidus*, have extreme depth ranges, having been recorded from the shelf, slope, and basin-floor regions. Both of these species have also been recorded from Baffin Bay.

It is noteworthy that records of *Ophiopholis aculeata* are so few in East Greenland waters. The species has an extremely wide distribution in both the Pacific and Atlantic, in boreal as well as arctic regions. Records indicate it is certainly tolerant of high arctic conditions.

## ACKNOWLEDGMENTS

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## STATION LIST: ARLIS II.

### BOTTOM SAMPLES CONTAINING ECHINODERMS

*Station 19*: Feb. 19, 1965. 78°00′N–09°53′W, 235 m. *Ophiopleura borealis*

*Station 44*: Feb. 23, 1965. 77°33′N–09°28′W, 275 m. *Gorgonocephalus eucnemis*

*Station 88*: Mar. 1, 1965. 77°01′N–11°05′W, 365 m. *Icasterias panopla*

*Station 102*: Mar. 3, 1965. 76°33′N–12°02′W, 275 m. *Ophioscolex glacialis*

*Station 111*: Mar. 4, 1965. No position, 240 m. *Ophiacantha bidentata*

*Station 131*: Mar. 8, 1965. 76°01′N–12°51′W, 270 m. *Ophiocten sericeum*, *Strongylocentrotus droebachiensis*

*Station 132*: Mar. 8, 1965. 76°01′N–12°51′W, 270 m. *Ophioscolex glacialis*, *Strongylocentrotus droebachiensis*

*Station 139*: Mar. 9, 1965. No position, 375 m. *Pontaster tenuispinus*, *Ophiacantha bidentata*. *Ophiopleura borealis*

*Station 150*: Mar. 12, 1965. 75°09′N–13°31′W, 205 m. *Pontaster tenuispinus*, *Ophiacantha bidentata*, *Ophiocten sericeum*, *Ophiopleura borealis*

*Station 159*: Mar. 13, 1965. 75°04′N–13°30′W, 205 m. *Ophiopus arcticus*

*Station 166*: Mar. 14, 1965. 74°53′N–13°36′W, 205 m. *Ophioscolex glacialis*, *Ophiacantha bidentata*, *Ophiopus arcticus*, *Strongylocentrotus droebachiensis*

*Station 173*: Mar. 16, 1965. 74°30′N–14°20′W, 205 m. *Poliometra prolixa*, *Hymenaster pellucidus*, *Ophioscolex glacialis*, *Ophiacantha bidentata*, *Ophiopus arcticus*, *Ophiopholis aculeata*, *Ophiura sarsi*, *Ophiocten sericeum*, *Strongylocentrotus droebachiensis*

*Station 180*: Mar. 17, 1965. 74°20′N–14°33′W, 250 m. *Poliometra prolixa*, *Ophiacantha bidentata*, *Ophiocten sericeum*

*Station 196*: Mar. 19, 1965. 74°09′N–14°53′W, 295 m. *Poliometra prolixa*

*Station 210*: Mar. 22, 1965. 73°27′N–16°02′W, 435 m. *Korethraster hispidus*, *Ophiacantha bidentata*, *Ophiopus arcticus*, *Strongylocentrotus droebachiensis*

*Station 222*: Mar. 24, 1965. 72°57′N–16°51′W, 315 m. *Ophiocten sericeum*

*Station 230*: Mar. 25, 1965. 72°50′N–16°44′W, 320 m. *Ophiacantha bidentata*

*Station 238*: Mar. 26, 1965. 72°46′N–16°41′W, 325 m. *Poliometra prolixa*

*Station 297*: Apr. 6, 1965. 71°58′N–17°32′W, 1615 m. *Ilycrinus carpenteri*

*Station 319*: Apr. 10, 1965. 71°50′N–17°25′W, 1635 m. *Ilycrinus carpenteri*, *Elpidia glacialis*

*Station 332*: Apr. 14, 1965. 69°45′N–19°20′W, 355 m. *Poliometra prolixa*, *Ophiacantha bidentata*, *Ophiopus arcticus*

*Station 390*: Apr. 25, 1965. 68°02′N–23°13′W, 1230 m. *Ophioscolex glacialis*

# Prespawning Adult Salmon Mortality Associated with a Fungus of the Genus *Dermocystidium*[1,2]

By Gilbert B. Pauley

*Biology Department, Battelle Memorial Institute*
*Pacific Northwest Laboratory, Richland, Washington*

## ABSTRACT

A serious outbreak of mortalities occurred among adult salmon held in the Priest Rapids Dam spawning channel in November 1965. Gross examination revealed small, white cysts on and in the gill filaments of otherwise normal-appearing fish. Approximately 25% of the 5000 mature adult salmon in the channel died prior to spawning from this disease caused by a fungus identified as the genus *Dermocystidium*. This is the first record of an epidemic among fish of North America associated with *Dermocystidium*. The primary cause of death appeared to be anoxia due to subacute inflammation and granulation of the gill tissue. A description of the histopathology associated with these parasites is given. *Chondrococcus columnaris*, a pathogen of warm-water fishes, was present as a secondary invader on the gill filaments of several salmon and an unknown fungal or yeast-like organism was present in the testes of the male fish examined.

## INTRODUCTION

In November 1965 a serious outbreak of mortalities occurred for "no apparent reason" among adult salmon, *Oncorhynchus tshawytscha*, held in the spawning channel at Priest Rapids Dam, Washington. Gross examination of these fish showed small, white cysts, measuring approximately 1 mm in diameter, on and in the gill filaments (Fig. 1). Otherwise, the fish appeared healthy.

Examination of these cysts led to the identification of a fungal parasite belonging to the genus *Dermocystidium*. Although an exact mortality figure is not available, it is estimated that approximately 25% of the 5000 adult fish held at Priest Rapids spawning channel died prior to spawning due to this disease. Several miles downstream from Priest Rapids Dam this disease organism was also observed in several adult chinook salmon held in the hatchery at the Biology Department of the Pacific Northwest Laboratories. However, there were no apparent deaths at this hatchery due to *Dermocystidium*. A parasite, similar to *Dermocystidium* upon gross examination, was observed on the gills of a few adult chinook salmon held in the McNary Dam spawning channel in the fall of 1964 (Fujihara, personal communication), but excessive deaths were not apparent.

Léger (1914) described *Dermocystidium branchialis* from cysts under the gill epithelium of the trout, *Trutta fario*. Dunkerley (1914) found this same species of *Dermocystidium* on *T. fario*, but failed to recognize it as a species different from *D. pusula* described by Pérez (1907). From cysts found on the

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gills of the pike, *Esox lucius*, Jirovec (1939) described *D. vejdovskyi*. The carp, *Cyprinus carpio*, was observed to have *D. koi* parasitic in cysts on the skin and in the muscle tissue, but not on the gills, with some associated inflammatory response by the host to this parasite (Hoshina and Sahara, 1950).

Reichenbach-Klinke (1950) described *D. percae*, which produces cysts on the fins, head, and skin of the perch, *Perca fluviatilis*. Two species of sticklebacks, *Gasterosteus aculeatus* and *G. pungitius*, were noted with cylindrical cysts of *D. gasterostei* covering their entire bodies, including the cornea of the eye, with some possessing cysts that penetrated deeply between the bones of the skull (Elkan, 1962). In their book dealing with the diseases of lower vertebrates, Reichenbach-Klinke and Elkan (1965) have briefly reviewed some of the previous work done with *Dermocystidium*.

*Dermocystidium salmonis* was noted from cysts located on the gills of a salmon *Oncorhynchus tshawytscha* taken from the Sacramento River, California (Davis, 1947). This was the first record of a North American species of *Dermocystidium*. The only other record of this genus from North America is *D. marinum*, described by Mackin et al. (1950) from the American oyster, *Crassostrea virginica*. The latter is the only species of this parasite that has either a marine or an invertebrate host and it has received widespread attention due to the economic value of the oyster and the ability of the fungus to cause catastrophic mortalities among oyster populations. This marine form of the parasite differs from the freshwater members of the genus by failing to form cysts in the host. Ray (1952, 1954) demonstrated a quick and reliable method for diagnosis of *D. marinum* in oysters, and also showed that *D. marinum* is definitely a fungus. Several marine mollusks, both bivalves and gastropods, have been reported to harbor infections of fungal parasites similar to *D. marinum* (Ray and Chandler, 1955).

An acount of the histopathology associated with *D. marinum* in oysters is given by Mackin (1951). However, no comparable description exists of the histopathological manifestations of this disease in any fish infected with *Dermocystidium*.

## MATERIALS AND METHODS

Wet mounts of cysts were made from approximately 50 fish and examined in the field. Bacterial and fungal streaks were made for laboratory culture and examination from all of the fish examined in the field. The medium used for bacterial culture contained 0.4% Bactotryptone, 0.04% yeast extract, and 1% agar, and was adjusted to *p*H 7.3. Czapek's modified media was used as the fungal media (Williams, 1958). Smears were made of fresh blood drawn from the heart of 15 fish. Samples of gill tissue and other organs from 15 fish were preserved in methanol for later histopathological examination. Tissue sections were stained with Mayer's hematoxylin and eosin, Giemsa, and Gridley's fungus stain. Giemsa was also used to stain the blood smears. Approximately 50% of the fish examined were alive. However, all of the fish examined, living or dead, had at least a light infection of *Dermocystidium*.

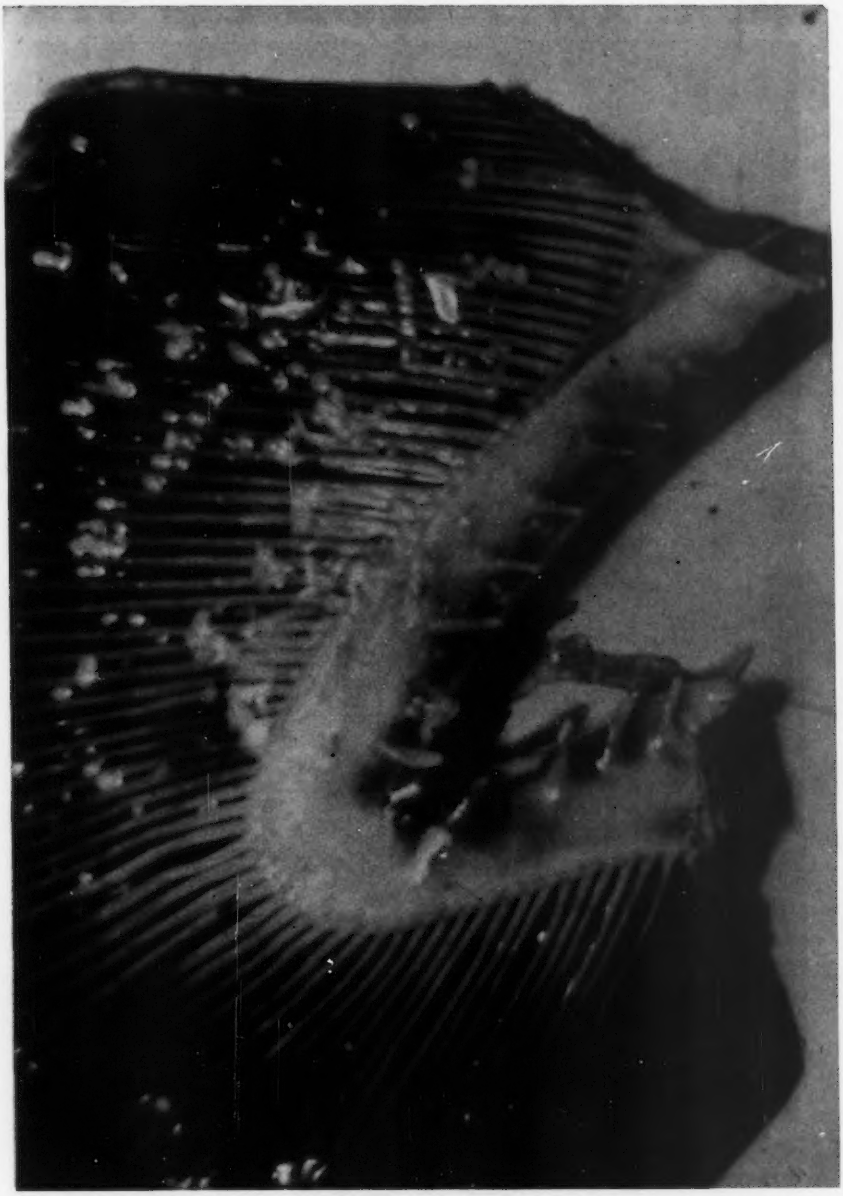

FIG. 1. Excised gill of a mature salmon, *Oncorhynchus tshawytscha*, showing heavy infection of the fungus parasite *Dermocystidium*. Approximately three times normal size.

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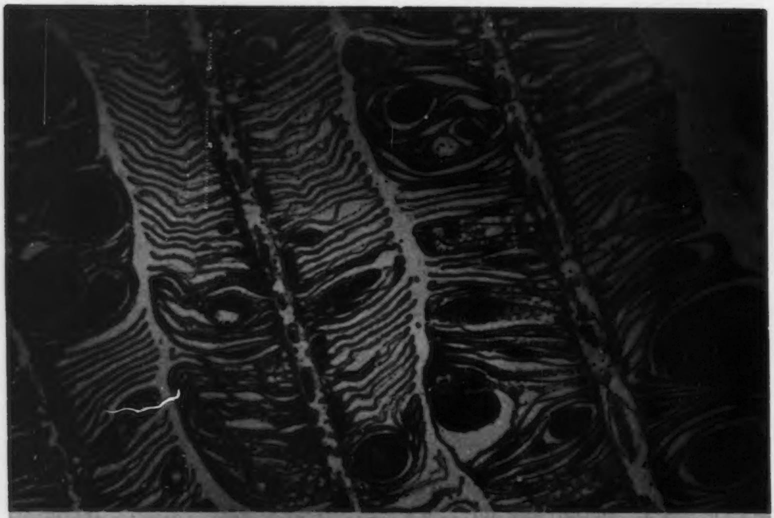


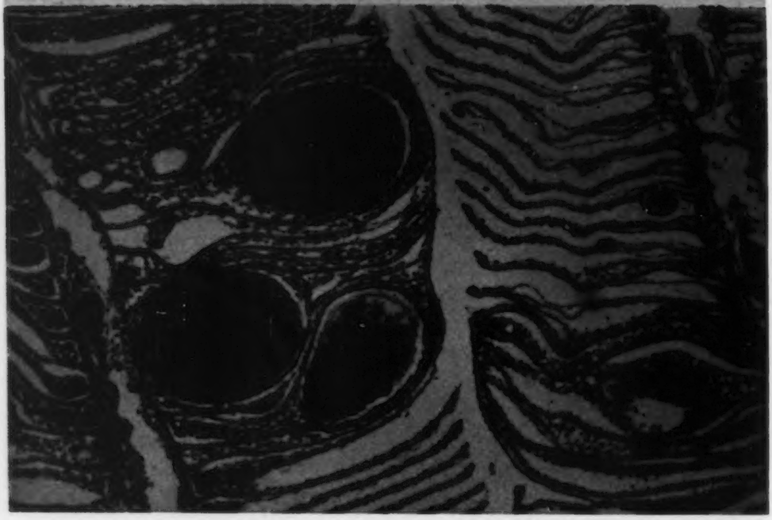
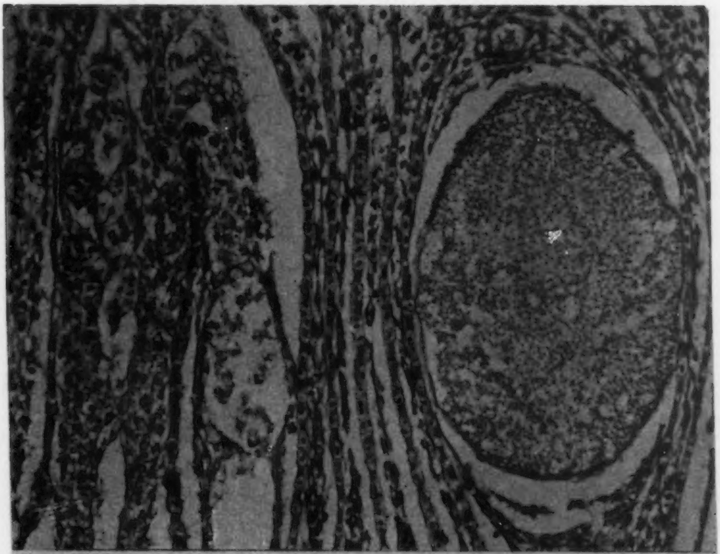


FIG. 2. (*Top*) Salmon gill heavily infected with *Dermocystidium*. Note the numerous different sized cysts. Giemsa. 30×.

FIG. 3. (*Bottom*) Salmon gill heavily infected with *Dermocystidium*. Note the three large cysts or colonies with mature cells and the small cyst at right containing immature "Dermo" cells. Giemsa. 60×.



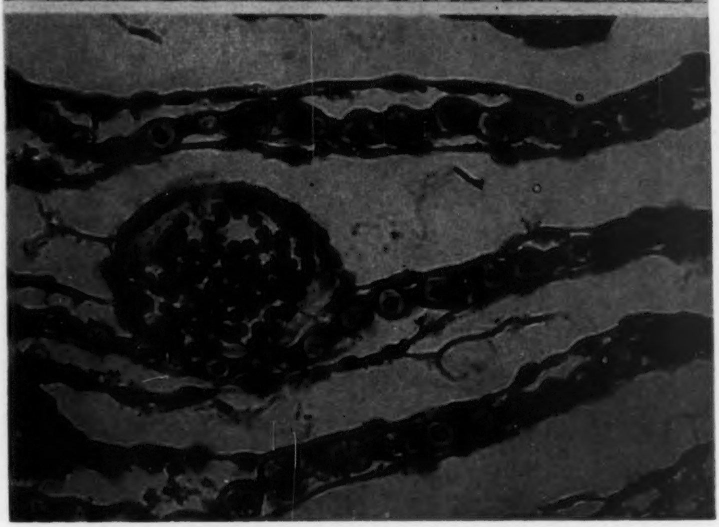
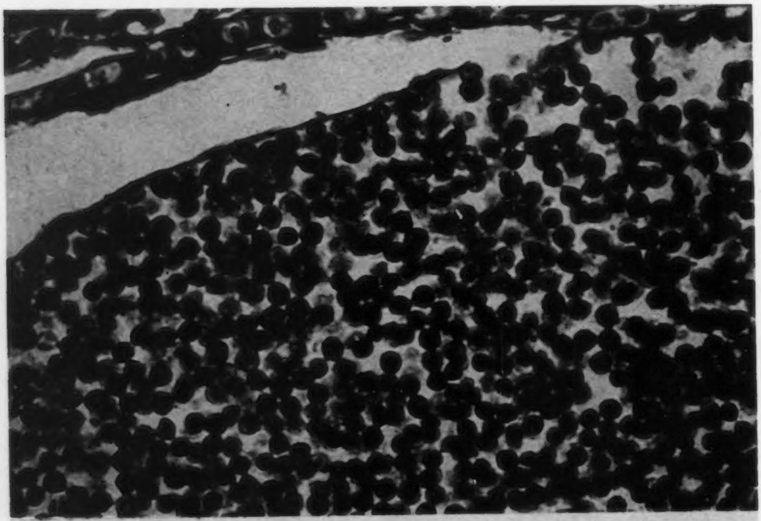


FIG. 4. (*Top*) *Dermocystidium* colony has caused near fusion of the gill filaments adjacent to it. Note the subacute inflammatory tissue among gill filaments at left. Hematoxylin and eosin. 155×.

FIG. 5. (*Bottom*) Small *Dermocystidium* colony containing immature "Dermo" cells. Giemsa. 390×.



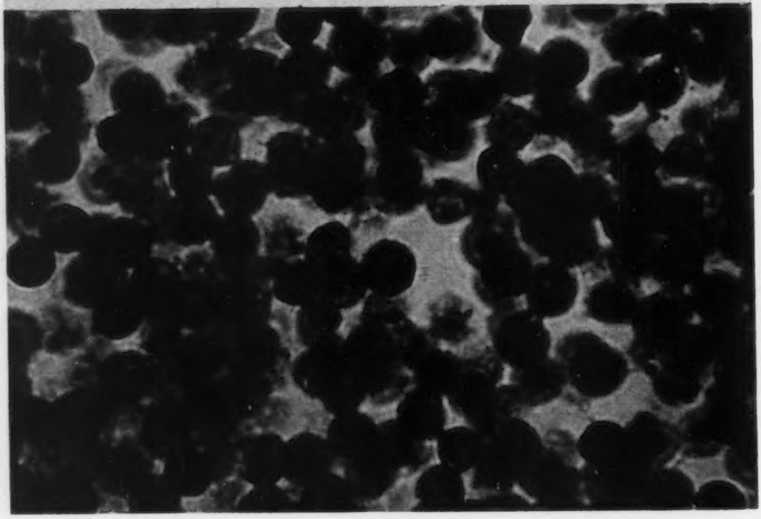
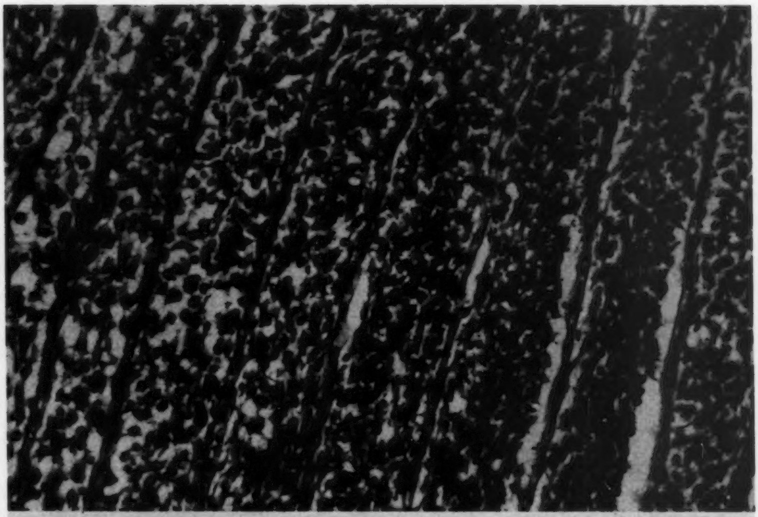


FIG. 6. (*Top*) A portion of a *Dermocystidium* colony showing individual "Dermo" cells. Giemsa. 390×.

FIG. 7. (*Bottom*) Higher magnification of individual *Dermocystidium* cells. Giemsa. 960×.



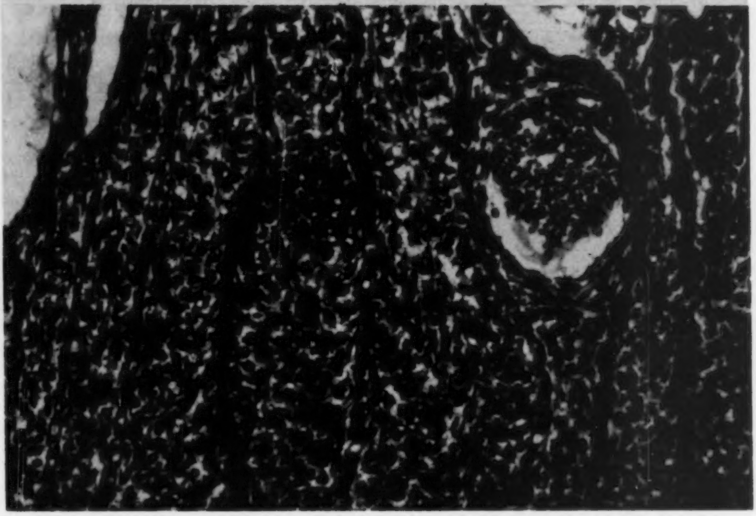


FIG. 8. (*Top*) Subacute inflammatory tissue among gill filaments of salmon infected with *Dermocystidium*. Note the gill filaments are still recognizeable. Giemsa. 155×.

FIG. 9. (*Bottom*) Granulation tissue among gill filaments and around one *Dermocystidium* colony. Note the distortion of the gill filaments which are nearly unrecognizeable. Giemsa. 155×.

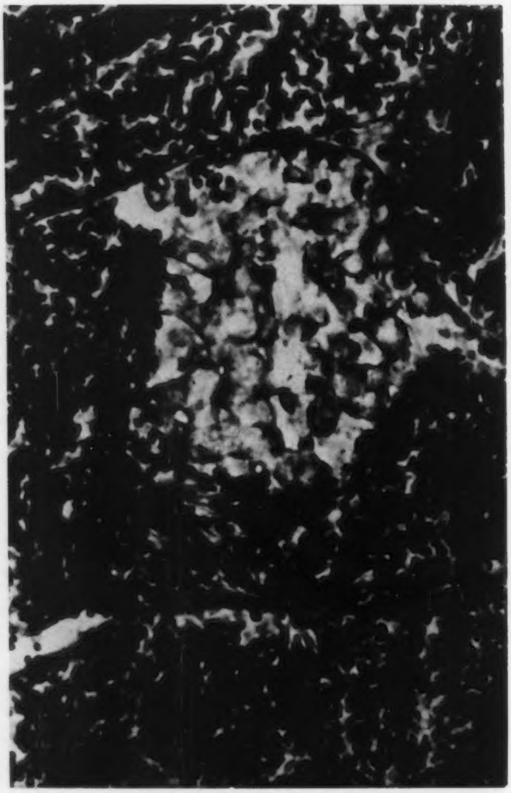


FIG. 10. Testes of a male salmon containing an unknown fungal or yeast-like structure. Note the apparent lack of a host response to this invader. Hematoxylin and eosin. 390×.

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## IDENTIFICATION AND DESCRIPTION OF THE PARASITE

From the wet mount slides examined in the field, the cysts were observed to contain a large number of single-celled organisms that possessed an eccentric nucleus and a large vacuole with an inclusion body. Confirmation of this morphology in tissue sections stained with hematoxylin and eosin or Giemsa (Fig. 6 and 7) led to the identification of this parasite as a member of the fungal genus *Dermocystidium*; probably, *D. salmonis* described by Davis (1947). This is the first recorded *Dermocystidium* epidemic among fish or other vertebrates from North America, as Davis' finding was from a single fish. Bacterial plates yielded some light growth of *Chondrococcus columnaris* as described by Ordal and Rucker (1944). These bacteria were also observed in tissue sections stained with Giemsa, as chain-like masses.

The gills of the salmon contained cysts of different sizes (Fig. 2 and 3) with a clear capsule wall which possessed several developmental stages of the parasite that varied in size from 5 to 8 μ. Many small cysts were observed containing only granular material. Some investigators believe these to be immature forms which later develop into mature spores (Léger, 1914; Broz and Privora, 1952). Because of the usual small size of these cysts, it is not certain from our preparations if this granular material is an immature form of the parasite, granular debris of necrotic parasites, or degenerating cysts. A few individual parasites were observed attached to the squamous epithelium of the gill. Davis (1947) observed individual *D. salmonis* mixed into the mucous of the gill, but not actually attached to the epithelium.

The spleen was the only other tissue examined that apparently contained stages of *Dermocystidium*. This organ contained numerous nonvacuolated cells, containing one to four nuclei, that resembled the immature spores drawn by Davis (1947).

## HISTOPATHOLOGY

Since very few details exist concerning the histopathology associated with *Dermocystidium* infections in vertebrate hosts, a description of the lesions accompanying this disease in salmon is given. Three major areas, the oral cavity, the gills, and the spleen in infected adult salmon, were found to have *Dermocystidium* associated histopathology. The testes of the five infected male fish were found to harbor an unknown parasite.

There was a distinct hydropic degeneration and associated hypertrophy of the squamous epithelium of the mouth and gill arches. There were many congested gill filaments, often with an accompanying area of hemorrhage between the filaments. An apparent edema of the dense connective tissue of the gills, gill arches, and mouth was present.

Cysts of varying size and possessing a clear, thin capsule were present among the gill filaments. The clear cyst capsules were apparently produced by the *Dermocystidium* colony. There was a subacute inflammatory reaction around these cysts with well-developed granulation tissue present containing many lymphocytes, monocytes, macrophages, and some granulocytes (Fig. 9). The epithelium of the gill filaments was hypertrophied and in some places it was hyperplastic. In many instances, the hyperplastic epithelial cells, along with macrophages and fibroblasts, filled in the space normally present between the gill filaments, resulting in a fusion of these filaments (Fig. 4 and 8). This fusion is briefly discussed by Davis (1947). The cysts contained various developmental stages of *Dermocystidium* with most of the larger cysts containing some of the typical vacuolated hypnospores with their characteristic volutin bodies. This stage is particularly easy to observe in Giemsa preparations, where the refringent volutin body is extremely noticeable and stained pink.

In the Giemsa stained sections, long, rod-shaped bacteria in filamentous chains were observed on isolated areas of the gills of the heavily infected fish. Only a very small number of these bacteria, identified as *Chondrococcus columnaris*, were observed on plates and then only on those smeared with gill scrapings that were heavily infected with *Dermocystidum*.

The spleens examined all showed an increased number of white blood cells in the blood vessels, and numerous nonvacuolated, multinucleated cells, suspected as being a stage of *Dermocystidium*. The normal architecture of the spleen was masked by congestion and an increased amount of fibrous connective tissue in the white pulp. Most spleens were also invaded by an unknown nematode which caused a granulation tissue response on the part of the host. In many instances, the veins and arteries of the spleen were occluded by this parasite; therefore, it is not known if the splenic effects were due to the nematodes or the multinucleate cells, thought to be stages of *Dermocystidium*. A synergistic effect of the two parasites cannot be ruled out.

The male salmon that were examined showed small, nodular, fungal, or yeast-like structures throughout the testes (Fig. 10). This structure replaced much of the area normally occupied by sperm, and in many instances there was some necrosis of the sperm directly adjacent to the parasite. However, there was no apparent host reaction to this structure. The sperm not in direct contact with the invader was normal and viable in appearance.

## DISCUSSION

According to Mackin (1962) all species of *Dermocystidium* are similar in that the hypnospore (1) develops a single, large vacuole in the cytoplasm which may contain a volutin body, (2) has a basophilic-staining cytoplasm which contains nucleus-like chromodia, and (3) has a large vesicular nucleus that is eccentric in position. The parasites observed in the salmon (*O. tshawytscha*) definitely possessed these characteristics and therefore were tentatively identified as belonging to the genus *Dermocystidium*. If this parasite is observed again among Columbia River salmon, positive identification should be made using a culture method similar to that of Ray (1952, 1954). The available information on the only member of this genus with a known life cycle (*D. marinum*) has been excellently reviewed by Mackin (1962). However, more information is needed on the life history of the *Dermocystidium* found in vertebrates.

The myxobacteria (*C. columnaris*) present on the gills appeared to be secondary invaders because they were only attached to tissue already heavily infected by *Dermocystidium* and were not present in large enough numbers to be suspected as the cause of death. The primary cause of death in the mature unspawned salmon appeared to be anoxia, resulting from subacute inflammation and fibrous and granulation tissue formed in response to the *Dermocystidium*.

It is thought that the yeast-like structure found in the testes may have located there because it either (1) was associated with the *Dermocystidium* infection and was possibly a stage in the life cycle of this parasite, (2) was a secondary invader following the primary "Dermo" infection, or (3) was a natural phenomenon accompanying sexual maturation. The latter reason seems the most feasible, since it did not evoke a host response. However, this structure is not mentioned by Colgrove (1966) in his paper dealing with histological changes that occur during sexual maturation in salmon.

Serious outbreaks of *Dermocystidium* have been very rare, especially in economically important fish; therefore, the literature provides no information on the treatment of this parasite. The infection rate of *D. marinum* has been shown to be definitely elevated by high temperatures and salinities (Mackin, 1962). If additional outbreaks of this disease occur among Columbia River salmon in future years, methods of regulating the temperature and changing the salinity of the water should be investigated as possible methods of control. Concentrated salt solution baths given to adult salmon at periodic intervals may be an economical and practical method of satisfactory control for this parasite.

## ACKNOWLEDGMENTS

The cooperative efforts contributed by Messrs D. E. Schoeneman, T. K. Meekin, A. C. Moser, and G. Tangen of the Washington State Department of Fisheries are gratefully acknowledged. Mr R. L. Westgard, Washington State Department of Fisheries pathologist, aided in identifying the causative agent as *Dermocystidium*. The author is indebted to Mr W. C. Renfro, Department of Oceanography, Oregon State University for the translations of the German literature cited. Messrs M. P. Fujihara and P. A. Olson, Research Scientists of the Pacific Northwest Laboratory, aided in the collection of samples. Technical assistance from Mr J. D. Maulsby is gratefully acknowledged. I am extremely grateful to Dr W. J. Clarke, Manager of the Pathology Section at the Pacific Northwest Laboratory, for his assistance and interpretation of many of the histopathological phenomena. Mr M. P. Fujihara cultured and identified the bacteria. The manuscript was critically reviewed by Drs R. E. Nakatani, W. J. Clarke, and Mr M. P. Fujihara.

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# Marking Pacific Salmon with Tetracycline Antibiotics[1]

BY DOUGLAS WEBER AND GEORGE J. RIDGWAY[2]

*U.S. Bureau of Commercial Fisheries*
*Biological Laboratory, Seattle, Washington*

## ABSTRACT

Diets containing tetracycline antibiotics were fed to hatchery populations of Pacific salmon. Subsequent examination of bone samples from these populations indicates that the tetracyclines are deposited permanently in the skeletal structure, are readily detectable after a period of rapid growth, and do not affect growth or survival. The most satisfactory and consistent marks were obtained by feeding 250 mg of tetracycline antibiotic per kilogram of body weight per day for 4 consecutive days.

## INTRODUCTION

IN THE WINTER OF 1887–88, the first hatchery-reared Pacific salmon (*Oncorhynchus* spp.) were released into the Columbia River system. From this beginning an extensive complex of salmon propagation facilities has evolved on the Pacific coast. These facilities are justified on the premise that artificial propagation aids in sustaining salmon populations. Little doubt exists that salmon hatcheries do contribute to the size of the catch and survival of the species, but the difficult problem of assessing the extent of this contribution still remains.

To assess hatchery contribution, hatchery-reared individuals must be distinguished from naturally spawned individuals. We suggested previously Weber and Ridgway, 1962) that tetracycline antibiotics may be used to mark and thus identify hatchery-reared fish. We demonstrated that in fish, antibiotics of the tetracycline series localize in areas of bone proliferation. To observe the deposited tetracycline, bone is illuminated with ultraviolet light which excites the antibiotic to fluoresce as a yellow line indicating the zone of bone calcification at the time of tetracycline administration (Fig. 1). The width of the line depends on duration of tetracycline administration and rate of bone formation; the intensity depends on dosage. Multiple lines may be formed by administration at suitably spaced time intervals.

This report describes first our method of marking Pacific salmon by oral administration of tetracycline antibiotics and the methods used in detecting the resultant mark. The successive sections concern the major objectives of our study: to determine retention time of marks, and to evaluate the effects of marking on growth and survival.

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[2] Present address (G.J.R.): U.S. Bureau of Commercial Fisheries, Boothbay Harbor, Maine.

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## MARKING WITH TETRACYCLINES

### BACKGROUND

The three main tetracyclines applied in this study are tetracycline (TC), oxytetracycline (OTC), and chlortetracycline (CTC). TC is considered the parent compound, and differs from OTC by an OH group, and from CTC by a chlorine. Two other commercially produced tetracycline homologues tested for marking quality were demethylchlortetracycline (DCTC) and N-pyrrolidinomethyl tetracycline. DCTC is similar to CTC, but lacks a methyl group; N-pyrrolidinomethyl tetracycline is TC with a methane-pyrrol ring attached.

#### DEPOSITION

All the tetracyclines appear similar in their property of deposition in the bone. Localization of tetracyclines in areas where calcification is taking place is believed to be the result of their ability to form complexes with the apatite surface calcium of newly forming bone tissue (Isben and Urist, 1964). Kelly and Byske (1960) concluded that, with the exception of a calcium chelate formation, the tetracyclines are deposited directly from the blood onto the bone surfaces in a chemically unaltered form. Tetracyclines do not deposit in normal cartilage and soft tissue, but do deposit in calcified cartilage of skeletal neoplasms (Milch et al., 1961) and soft tissue tumors (Johnson, 1964).

#### ABSORPTION AND FLUORESCENCE

Detection of tetracyclines in bone depends upon their fluorescent property. Fluorescence results when a molecule absorbs light energy which raises the molecule to an excited state. Upon return to its fundamental state the molecule emits light energy (fluorescence) — usually of a longer wavelength than the light absorbed. In aqueous solution the tetracyclines display two maximum absorption peaks in the ultraviolet range, one at 270 $m\mu$ and the other at 360 $m\mu$. Hattner and Frost (1962) reported that the 360 $m\mu$ absorption peak of TC and DCTC is shifted to 440 $m\mu$ upon deposition in human bone. We have no evidence for a similar shift by any of the tetracyclines after deposition in fish bone.

The visible yellow fluorescence of tetracycline deposited in fish bone results from ultraviolet absorption in the 360 $m\mu$ range; activation of the 270-$m\mu$ absorption peak does not produce visible fluorescence. Harris et al. (1962) noticed differences in the fluorescent spectra of OTC (yellow-green) and CTC (yellow) deposited in canine bone. In fish bone all the tetracycline fluorophores we studied were yellow except for the yellow-orange fluorophore of DCTC.

### MATERIALS AND METHODS

#### FISH

Four species of Pacific salmon (coho, *Oncorhynchus kisutch*; sockeye, *O. nerka*; chinook, *O. tshawytscha*; chum, *O. keta*) and rainbow trout (*Salmo gairdneri*) were used in our studies. At time of marking, the fish weighed 2–40 g, but the majority of experiments were performed on fish of 5–10 g. Control groups were maintained as a check on short-term mortality and acceptability of diets.

### TETRACYCLINES

Most of our studies were conducted with the following commercially available veterinary forms of CTC and OTC: water-soluble CTC and OTC with glucosamine at 25 g per pound; and CTC and OTC in feed mix at 40 and 50 g per pound respectively. Pure TC, OTC, and CTC, and human-consumption grades of DCTC and N-pyrrolidinomethyl tetracycline were used to determine if differences existed between deposited fluorophores, and in experiments where we wanted to eliminate the effects of large quantities of carrier material typical of the veterinary forms.

### DIETS AND APPLICATION

The antibiotics were combined without special mixing procedures in three basic diets: commercial dry mash which was pelletized or crumbled after mixing; wet diet consisting of liver and spleen; and Oregon moist pellet as described by Hublou (1963). The proportions of antibiotic depended on the form used, dosage applied, and the percentage of body weight fed per day. To ensure complete ingestion of antibiotic, the fish were not fed for 2 days before each experiment, and the amount of food given was below the normal hatchery requirements for the prevailing water temperature and size of fish. Throughout all experiments the water temperatures were maintained between 10 and 15 C.

In force-feeding studies, gelatin capsules (No. 4) were filled with the desired quantity of antibiotic and orally administered with a 5-mm diam cork borer.

### EVALUATION OF MARK

At the termination of each feeding experiment, 25 or more fish were selected at random and examined by fluorescent microscopy to determine effectiveness of marking. No analytical procedure is as sensitive as this visual technique. We assigned marking quality in relation to a standard or "good" mark resulting from intraperitoneal injection of 100 mg of OTC or CTC per kilogram of body weight. Marks resulting from feeding were divided into four categories based on the above standard: good — clear and intense; fair — clear but not intense; poor — present but neither clear nor intense; none — no mark evident.

## Dose and Duration of Feeding

The results of feeding varying dosages of pure CTC in a dry pellet diet to sockeye salmon fingerlings are given in Table I. The basic relation of dose and resultant mark in this series is typical of our observations with the other antibiotics and species of fish. We found that three generally consistent factors relate dose, days fed, and marking quality. First, daily dosages are at least as important as the cumulative dose. With a cumulative dose of 1000 mg/kg, feeding less than 250 mg per day yields a large proportion of poor marks, whereas feeding 250 mg or more per day results in a majority of good marks. Second,

TABLE I. Relation of dose, days fed, and quality of mark in fingerling sockeye salmon of 5–10 g body weight. Antibiotic tested: pure CTC mixed in a dry pellet diet.

| Dose per day (*mg/kg body weight*) | Consecutive days fed | Total dose (*mg/kg body weight*) | Quality of mark | | | |
|---|---|---|---|---|---|---|
| | | | Per cent of sample in each category | | | |
| | | | Good | Fair | Poor | None |
| 25 | 10 | 250 | 0 | 12 | 76 | 12 |
| 50 | 5 | 250 | 0 | 8 | 60 | 32 |
| 50 | 10 | 500 | 0 | 44 | 56 | 0 |
| 100 | 10 | 1000 | 4 | 68 | 28 | 0 |
| 125 | 8 | 1000 | 10 | 68 | 22 | 0 |
| 200 | 5 | 1000 | 32 | 49 | 14 | 5 |
| 250 | 4 | 1000 | 50 | 50 | 0 | 0 |
| 333 | 3 | 1000 | 68 | 18 | 12 | 2 |
| 500 | 2 | 1000 | 60 | 30 | 0 | 10 |
| 500 | 3 | 1500 | 78 | 18 | 4 | 0 |
| 500 | 4 | 2000 | 93 | 7 | 0 | 0 |
| 400 | 5 | 2000 | 89 | 11 | 0 | 0 |
| 667 | 3 | 2000 | 79 | 19 | 1 | 1 |
| 1000 | 2 | 2000 | 55 | 20 | 10 | 15 |

there is a limit in daily dosages of antibotic above which the resultant mark is not improved; orally, this dose is 500 mg per kilogram per day (intraperitoneally, it is 100 mg per day). Third, when the dose is sufficient the percentage marked is directly related to the number of days fed. Individual daily feeding response varies within a group of fish, and 100% marking is not achieved unless feeding is continued several consecutive days. Feeding for 2 or 3 consecutive days usually gives marks of varying intensity in 85–95% of the fish, whereas feeding for 4 or 5 consecutive days gives marks of uniform intensity in 96–100% of the individuals.

A total dosage of 1 g of tetracycline antibiotic per kilogram of body weight divided into doses of at least 250 mg/kg and given over 3 or 4 consecutive days normally produces a good mark. Several additional factors, however, may improve or diminish the effectiveness of marking.

### CALCIUM CONTENT OF DIET

The high oral dosage we found necessary for satisfactory marking probably indicates that fish are inefficient in absorbing tetracyclines from the gut. There is evidence that some of this inefficiency may be the result of the presence of calcium in the diet.

Calcium and other divalent and trivalent metallic cations will chelate with tetracyclines and reduce absorption of the antibiotic. This reduction was found

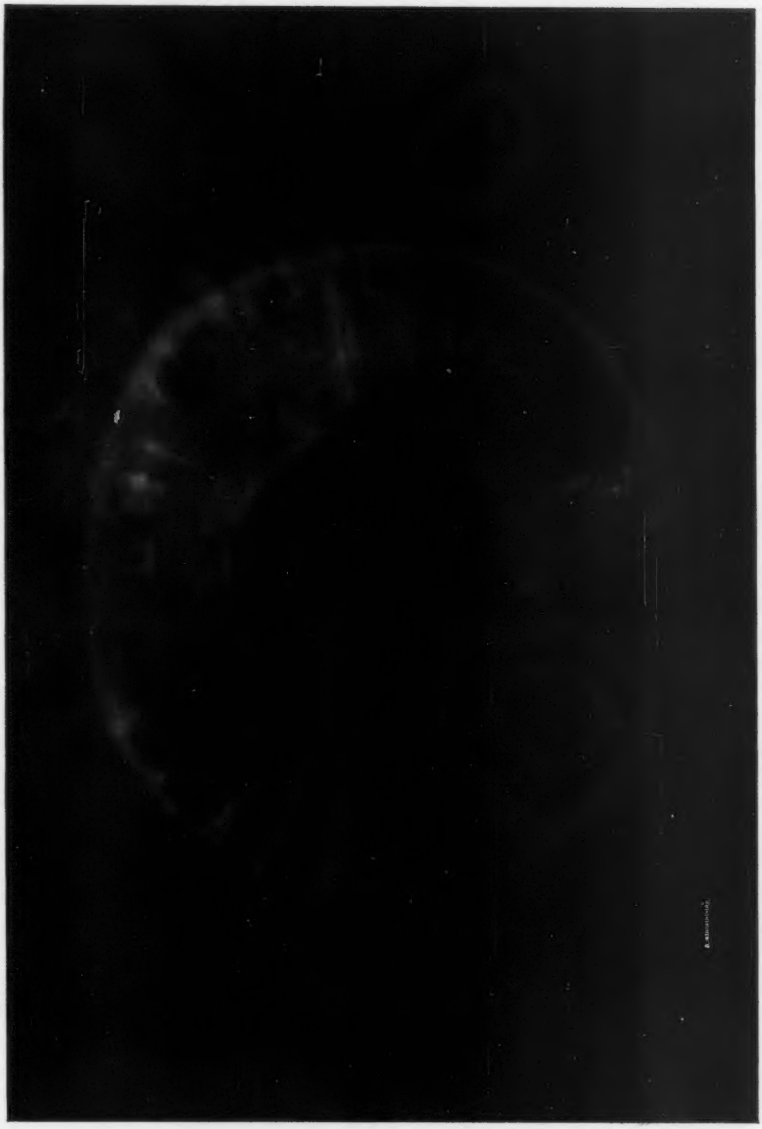

FIG. 1. Photomicrograph (35×) of a tetracycline-marked vertebral centrum from an adult sockeye salmon (*Oncorhynchus nerka*). This fish was marked with oxytetracycline as a fingerling in 1962 and recovered in 1965.

by Donovan et al. (1962), who noted that chickens fed poultry rations containing 0.3% dietary calcium had significantly higher blood levels of antibiotic than when fed higher levels of calcium. Studies by Westgate et al. (MS, 1964) on fingerling chinook salmon indicated that TC and OTC incorporated in a low-calcium (0.25%) Oregon pellet diet produced an appreciably better mark than the same antibiotic dose fed in standard Oregon pellet (2.0% calcium).

### POTENTIATORS

The introduction of a potentiator, a compound which enhances the action of another compound, has been shown to increase the blood level of tetracycline in birds and mammals (Gittinger and Wiener, 1960; Harms et al., 1963). In one set of experiments we mixed pure CTC with two common potentiators, glucosamine and terephthalic acid, and force-fed single doses of these tetracycline complexes to fingerling sockeye salmon. The intensity of deposited fluorophore definitely increased in the group given glucosamine, upgrading the mark from fair to good (Table II). Terephthalic acid, though not as effective as glucosamine, also improved the mark.

### FISH SIZE

Osteogenesis in Pacific salmon and rainbow trout has advanced sufficiently for uptake of tetracycline when the yolk sac is completely absorbed (called the "button-up" stage). At this stage the ribs and vertebral plates surrounding the notochord are beginning to form, and tetracycline may be incorporated on their growing surfaces. From button-up through the fingerling stage there is an increase in the quantity of bone matrix undergoing calcification, and thus, an increase in the number of available sites for tetracycline deposition.

The consequence of increasing calcification on tetracycline deposition is observed by the upgrading of marking quality coincident with administration of similar doses of tetracycline at progressively larger sizes. Jones and Steel (MS, 1965) made multiple marks on coho salmon fingerlings of various sizes by

TABLE II. Results of force-feeding fingerling sockeye salmon (weight range 30–35 g) different single doses of CTC mixed with glucosamine and terephthalic acid (TPA).

| Dose CTC (*mg/kg body weight*) | Number of fish | Dose of potentiator (*mg/kg body weight*) | | Quality of mark | | |
|---|---|---|---|---|---|---|
| | | Glucosamine | TPA | Good | Fair | Poor |
| 300 | 8 | Control | Control | 1 | 7 | – |
| 300 | 7 | – | 4.0 | 2 | 5 | – |
| 300 | 7 | 24.8 | – | 5 | 2 | – |
| 250 | 5 | Control | Control | – | 3 | 2 |
| 250 | 5 | – | 8.0 | – | 5 | – |
| 250 | 5 | 12.0 | – | 2 | 3 | – |

repeatedly feeding TC in a low-calcium Oregon pellet. TC fed at a dosage of 1 g/kg over 4 days at a count-weight of 290 per pound resulted in a poor mark, whereas the same dosage and duration of feeding at count-weights of 100, 50, and 25 per pound gave fair marks. Samples of the cleithrum from these multiple-marked fish after 7 months of oceanic life "showed 28% marked from feeding at 290 per pound, 97% from feeding at 100 per pound, and 100% from feeding at 50 and 25 per pound" (Jones, MS, 1965).

The smallest fish we marked and subsequently recovered after a period of oceanic life were chum salmon. These fish were at a count-weight of 220 per pound when fed a total dose of 2 g/kg of OTC with glucosamine mixed in a dry pellet diet given over 5 consecutive days. The marking quality was fair to good both at release in 1963 and at recovery in 1965.

### TETRACYCLINES

A 300-mg/kg dose of each of the pure forms of TC, OTC, CTC, and human-consumption grades of DCTC and N-pyrrolidinomethyl tetracycline was force-fed to 10-g sockeye, coho, and chinook salmon. The resultant fluorescence of the deposited tetracyclines was of equal intensity with the exception of DCTC, which was noticeably greater. It has also been reported by Hattner and Frost (1962) that, gram for gram, DCTC deposited in mammalian bone has a more intense fluorescence than other tetracyclines.

We found DCTC identical in acceptability and lack of toxicity to the other tetracyclines, but did not conduct major feeding trials with it because this antibiotic is not available in low-cost veterinary grades. N-pyrrolidinomethyl tetracycline, which is highly soluble, did not differ in deposition or efficiency of marking from TC, OTC, and CTC.

## Acceptability of Antibiotic Forms

The acceptability of diets containing antibiotics was tested on rainbow trout and sockeye and chinook salmon. In these tests the experimental and control groups were fed 3 times a day for 3 or 4 consecutive days; feeding responses of the associated control groups were excellent.

Most veterinary forms of antibiotic were accepted at high levels in the three basic diets. Diets containing up to 50% water-soluble forms of CTC and OTC with glucosamine were readily consumed by all species.

Experiments were conducted on the relation between acceptability and percentage concentration of antibiotic feed mixtures incorporated in the diet (Table III). At low concentrations commercial feed mixtures containing either CTC or OTC are acceptable. CTC in feed is acceptable to sockeye salmon and rainbow trout when it constitutes less than 40% of the diet, but chinook salmon tend to reject CTC feed mixtures when it exceeds 20% of the diet. Diets containing OTC in feed gave varied results; most experiments indicated a low level of acceptance. Other experiments also indicated low acceptance but they were terminated after 2 days. They are not included here because unacceptable antibiotic forms are usually accepted during the first and often

TABLE III. Results of experiments on acceptability of CTC and OTC in feed mixtures. A minimum of 100 fingerling salmon or rainbow trout were used in each experiment.

| Species | Diet | Active ingredient (*mg/kg/day*) | Days fed | Acceptability | Per cent body weight fed/day | Per cent antibiotic feed mix in diet |
|---|---|---|---|---|---|---|
| *CTC (40 g/pound of feed)* | | | | | | |
| Sockeye | Dry | 250 | 4 | Good | 2.0 | 14.2 |
| Sockeye | Dry | 500 | 4 | Good | 2.0 | 27.4 |
| Sockeye | Ore. moist | 500 | 4 | Good | 2.0 | 30.0 |
| Sockeye | Dry | 700 | 3 | Good | 2.0 | 32.3 |
| Sockeye | Dry | 1000 | 4 | Poor | 2.0 | 56.8 |
| Rainbow trout | Dry | 1000 | 4 | Good | 3.0 | 28.0 |
| Rainbow trout | Dry | 2000 | 4 | Poor | 3.0 | 56.6 |
| Chinook | Ore. moist | 500 | 4 | Good | 5.0 | 13.0 |
| Chinook | Ore. moist | 500 | 4 | Poor | 2.0 | 28.5 |
| *OTC (50 g/pound of feed)* | | | | | | |
| Sockeye | Dry | 250 | 4 | Good | 2.0 | 11.8 |
| Sockeye | Dry | 300 | 4 | Poor | 2.0 | 16.0 |
| Sockeye | Dry | 500 | 3 | Poor | 2.0 | 22.7 |
| Sockeye | Ore. moist | 500 | 4 | Fair | 2.0 | 23.0 |
| Chinook | Ore. moist | 250 | 4 | Fair | 2.0 | 11.8 |
| Chinook | Ore. moist | 500 | 4 | Poor | 2.0 | 23.0 |
| Rainbow trout | Dry | 700 | 3 | Fair | 3.0 | 30.0 |
| Rainbow trout | Dry | 1000 | 4 | Poor | 3.0 | 45.5 |

second day of feeding; only after the third consecutive day of feeding is there definite rejection or acceptance of the diet.

We used water-soluble OTC with glucosamine in our major marking studies; not only was it acceptable at all levels of feeding, but it produced consistently good marks. CTC in feed mixtures, though acceptable at dosages required for marking, was not consistent in marking quality. This inconsistent marking may have been caused by either high levels of calcium in the diet or variations in condition of the fish.

## DETECTION OF MARKS

### EQUIPMENT

Detection of tetracyclines in bone requires microscopic examination with ultraviolet illumination. The light source used in the laboratory was a 200-w mercury-vapor arc lamp with UG-1 and BG-12 exciter filters. Ultraviolet-excluding objectives and a Wratten 2A eyepiece filter were used to absorb ultraviolet light, but allow passage of visible light through the ocular lenses. Three- and six-power objectives in combination with a 10-power ocular are sufficient for detection of deposited fluorophore.

Examination of samples in the field required portable equipment which included: microscope illuminator with 100-w mercury-arc lamp and transformer; blue pass exciter filter, BG-12 or Corning 5113; ultraviolet pass exciter filter, UG-1 or Corning 5840; and a Wratten 2A microscope eyepiece filter, ¾-inch diam, for a standard monocular microscope.

## Treatment of Samples

### FINGERLINGS

After administration of tetracyclines a general tissue fluorescence persists for several days. Even though deposition in bone is immediate, it is advisable to delay examination at least 1 week to allow for clearance of unbound antibiotic from the tissue and, more important, for additional bone growth. The contrasting bluish autofluorescence of unmarked bone on both sides of the deposited fluorophore allows better definition and evaluation of the quality of the mark. Some initial confusion may result from fluorescence of lipofuscin (yellow) and porphyrins (red). These tissue components, however, do not have the distinctive color characteristics or distribution of tetracycline-induced fluorescence.

Samples which were to be retained for a period of time before observation were frozen. Since normal illumination has a photolytic effect on deposited fluorophore, frozen samples were thawed and cleaned only immediately before observation. Flesh was removed from the bones with boiling water or hot tap water, and the cleaned material kept moist until examination. In most species of fingerling salmon and trout, the marks on the cleithrum or an anterior rib are a reliable index for determining the effectiveness of marking. In chinook salmon fingerlings, however, evaluation of a mark by using an anterior rib is difficult because the flange at the proximal end of the rib is not well developed.

### ADULTS

Detection of tetracycline in skeletal tissue requires that the sample be transparent to the exciting ultraviolet wavelengths and the visible light given off by the deposited fluorophore. These requirements are easily met in fingerlings, since most skeletal components are thin and transparent. As growth proceeds, however, changes are induced in the skeletal tissue by overlying bone growth and remodeling which impose limitations on the selection of bones suitable for sampling. Sectioning and grinding of bone samples to expose the tetracycline mark is slow and yields inconsistent results.

Bone samples were taken from coho salmon when they returned as "jacks" (precocious males averaging 1.4 kg) in 1963 and as adults in 1964; they had been marked in 1962 as fingerlings (average weight, 30 g). The gill rakers and vertebral arches of returning jacks were marked, but in adults these structures had been modified by bone reabsorption, and the tetracycline was no longer detectable. The hypurals and cleithrum and the epihyal, ceratohyal, and several other bones of the head become progressively more ossified with increasing size; though the mark in these bones was evident in jacks, the overlaid porous bone obscured the mark in adults. In the vertebral centrum, ribs, and entopterygoid, the deposited tetracycline was easily detected in both jacks and adults.

We also examined adult sockeye, chinook, and chum salmon which were marked with tetracycline as fingerlings, and found the vertebral centrum to be the most reliably consistent bone for detection of deposited fluorophore. Easy detection of marks in the centrum is a result of the growth pattern; no additional bone is laid down over previously existing calcified areas. The branchiostegals and opercular bones, though often well marked in fingerlings, do not retain tetracycline in detectable amounts in adults.

Flesh and cartilage may be removed from the bones of adult salmon with boiling water or hot tap water, but it is more efficient and effective to immerse the bones several hours in a constantly agitated solution of 2% trypsin (1:250 activity). After they are cleaned, bone samples from jack and adult salmon must be kept moist until examination is complete, because dehydration induces opaqueness and radically reduces detection of the deposited fluorophore.

As mentioned earlier, normal illumination and ultraviolet light have a photolytic effect on deposited tetracycline fluorophore. In preliminary studies we found that the scales of fish took up and retained tetracycline. Under laboratory conditions where fish were not exposed to direct sunlight, scale fluorescence persisted at least 13 months; but when fish were retained in outside ponds, scale fluorescence was evident for only 2 or 3 days after completion of feeding. The examination of a scale may be useful, however, for an immediate check on effectiveness of marking.

## PERMANENCE OF TETRACYCLINE MARK

A study designed to determine retention and ease of detection of deposited tetracycline was undertaken at the Oregon Fish Commission's Klaskanine Hatchery near Astoria, Oregon. In March 1962 we fed antibiotic to one-sixth of the 1960 year-class of coho salmon at the Klaskanine Hatchery (205,000 fish). OTC with glucosamine mixed in a standard wet diet was fed at a level of 500 mg/kg body weight per day on 2 consecutive days. Cleithra removed from 196 fish 2 days after feeding indicated that 94% of the fish were marked.

The coho salmon marked with OTC were the last fish released from the hatchery. At release they weighed 15 to the pound, identical in weight with unmarked fish released during the preceding 6 weeks. Since 94% of the coho salmon fed OTC were marked, we should expect 17% of all 1960 year-class coho salmon returning to the Klaskanine Hatchery to marks (assuming that the deposited tetracycline was retained, survival of marked and unmarked fish was equal, and straying minimal).

A fraction of the releases returned to the hatchery as jacks after 7 months of oceanic life; 10% of these returnees were sampled for tetracycline marks. In the first 500 fish taken, both the cleithrum and an anterior rib were removed. Intensity and clarity of marks on both structures were comparable; further sampling consisted of anterior rib bones only. In a sample of 1602 jacks, 22% bore a tetracycline mark — or about 5% more than expected.

Beginning in mid-September 1963, 1½ years after release of the marked fingerlings, the commercial fishery at Astoria was sampled. We hypothesized that some of the adults passing through the commercial fishery were of the 1960 year-class — reared at, and returning to, the Klaskanine Hatchery. The Oregon Fish Commission sampled 22% of the coho salmon in the commercial catch by removing the entopterygoid (a paired bone in the roof of the mouth beneath the eye that is not heavily ossified in adults; removal causes no observable mutilation of marketable fish). In 30 marked adult coho salmon the entopterygoid, anterior rib, and vertebral centrum were compared. The entopterygoid was satisfactory in each fish for determining the presence of a tetracycline mark. Of 822 adults from the commercial fishery, 17% were marked. We attributed the lower percentage of marked fish in the fishery samples (compared with the 22% return of jacks in the previous year) to intermingling of unmarked coho salmon from other areas.

By the end of the commercial fishing season, some of the adult coho salmon which had passed through the fishery were already entering the holding troughs at the Klaskanine Hatchery. At the hatchery, we sampled an anterior rib from 10% of the returning adults; of 437 examined, 21.3% were marked—about the same percentage as in returns of jacks in 1962. The percentages of marked adult males and females were not significantly different: 21.6% of 306 females and 20.6% of 131 males were marked.

Among the adult fish sampled at the Klaskanine Hatchery, the 4% increase in marked fish between release and return was probably due to a difference in survival. It is possible that the tetracycline curtailed a disease, or the time of release may have been more favorable for survival. If the tetracycline mark were not retained permanently, or if we were not able to detect the mark adequately, we would have expected a mark recovery of less than 17%.

We also tested the retention of deposited fluorophore after the coho salmon had spawned and died to determine if marked salmon could be identified during surveys on the spawning grounds. Six carcasses of coho salmon which were found to be marked were staked out in a stream in mid-November 1963. In early February 1964, bone tissue from these fish was examined for the tetracycline mark. Fluorescence was observed in the vertebrae in all six fish, and in three fish the anterior rib, though mostly decalcified, showed evidence of deposited fluorophore.

## SURVIVAL OF MARKED FISH

### SHORT-TERM EFFECT

Tetracyclines are recognized to have a low degree of toxicity for man and other animals, and we observed no distress or mortality after feeding the antibiotics in any of our laboratory or hatchery studies. In one experiment on 25 rainbow trout we fed 5200 mg of pure OTC per kilogram of body weight over 3 consecutive days, and in another study on 1000 chinook salmon fingerlings, we fed 1600 mg of pure OTC per kilogram of body weight per day for 5 consecutive days. In neither experiment was there evidence of toxicity or noticeable changes in behavior.

Studies on humans and other animals (Dessau and Sullivan, 1961; Welch, 1954) have given evidence that high oral doses may cause temporary gastrointestinal irritation. These effects are probably not manifestations of the antibiotic, but expressions of the effects of tetracyclines on the intestinal flora. According to Margolis (1953), fish do not have common bacterial commensals in the intestine, but "... the presence of bacterial flora depends solely on the recent intake of food and the degree of contamination of the food and water." This difference may well account for the absence of any adverse behavior in fish that were fed large quantities of tetracycline.

To evaluate short-term mortality after feeding OTC to sockeye salmon fingerlings, fish from the same hatchery lot (i.e. fertilization, hatching time, and handling procedure were similar) were distributed among 12 ponds (6 test and 6 control). In mid-July 1962, the number of fish in each pond was estimated. During 4 days in mid-August, fish in the six test ponds were fed 2 g/kg body weight of OTC with glucosamine in a dry pellet diet. The numbers of fish in each pond were again estimated in mid-October. In addition a record of daily mortality by pond was maintained throughout the study. Two estimates of mortality were derived from these data: one based on the daily mortality count and the other on differences in the estimated numbers in each group before marking with tetracycline and 2 months after marking. The daily mortality counts indicated a slightly, but not significantly, higher mortality for the fish fed tetracycline (Table IV). Even before feeding tetracycline, however, mortality in the test group was higher than in the control group. Mortality based on

TABLE IV. Short-term mortality of sockeye salmon fingerlings fed oxytetracycline (OTC), based on population estimates and on direct counts of dead fish.

| Date (1962) | Control | | Test | |
|---|---|---|---|---|
| | Population estimate | Observed mortality | Population estimate | Observed mortality |
| July 15 | 381,812 | | 381,301 | |
| August 4–11 | | 429 | | 544 |
| August 12–15[a] | | 100 | | 112 |
| August 16–23 | | 277 | | 225 |
| August 24–31 | | 191 | | 215 |
| September 1–30 | | 541 | | 602 |
| October 1–16 | | 569 | | 613 |
| October 16 | 347,644 | | 351,010 | |
| Total mortality | 34,168 | 1,678 | 30,291 | 1,785 |

[a] A total of 2 g of OTC per kg body weight was fed to the test group from August 12 to 15, 1962.

estimates of the number in each pond at two intervals was 9.0% in the control group and 8.0% in the test group. These percentages represent more reliable estimates of mortality than the daily count because the daily count did not include the many unobserved losses that presumably resulted from cannibalism or bird predation.

### LONG-TERM EFFECT

We initiated a field study in the summer of 1962 to determine if feeding and resulting deposition of tetracycline affected long-term survival. For this study we used sockeye salmon of the 1961 year-class reared at the Leavenworth National Fish Hatchery.

Leavenworth Hatchery is in the State of Washington just east of the Cascade Mountains. Sockeye salmon reared at Leavenworth are released into nearby Lake Wenatchee. After residing there for 7 months, the smolts migrate down the Wenatchee River and into the Columbia River.

Before marking with tetracycline, one-third of the sockeye salmon in each of 30 holding ponds were marked with an adipose-maxillary clip combination. After the fish had attained a count-weight of 125 per pound, we fed OTC with glucosamine and vitamin D (2 g of OTC per kilogram of body weight) in a low-calcium pelletized diet to the fingerlings in 10 of the 30 holding ponds for 4 consecutive days. A sample of 550 fish taken 2 months later from the 10 ponds indicated that 100% of the fish had been marked with the tetracycline.

At release, the entire hatchery production of sockeye salmon was randomly sampled with a 10-pocket sampler. One pocket of the sampler was closed, retaining 10% of the fish. The 10% retained were sampled again. The resulting 1% were then examined to estimate the numbers released in the following categories: 202,000 double marked with OTC and an adipose-maxillary clip; 425,900 OTC-marked only; 369,800 clipped only; and 779,000 unmarked.

In the fall of 1962, the sockeye salmon fingerlings were released in Lake Wenatchee; the following spring they migrated from the lake with naturally spawned sockeye smolts. We collected 2020 sockeye smolts at the lake outlet from April 15 to May 28, 1963. An anterior rib was removed from each fish, and they were checked for clips, measured, and weighed.

Our comparison for determining the effect of OTC on survival is between fish with a double mark (OTC plus clip) and those with clip only. Since both groups were clipped this comparison would show only the effect of tetracycline. Of 748 clipped fish recovered, 34.1% were double marked. At time of release from Leavenworth Hatchery, 35.3% of the clipped fish were double marked. Point estimates and confidence intervals for the percentage of double-marked fish at release and recovery are given in Table V. If administration of tetracycline did influence survival, we would expect the percentage of double marks to be significantly different from 35.3%.

The 1961 year-class of sockeye salmon released from Leavenworth Hatchery was sampled again when the fish returned as adults in the fall of 1965. At that time we removed a vertebra from each of 2050 caught in the commercial

TABLE V. Percentage double marked at release and recovery, and 95% confidence intervals around these percentages, for sockeye salmon marked at Leavenworth Hatchery in 1962, and recovered in 1963 and 1965.

| | Sample size | Percentage double marked | 95% confidence intervals |
|---|---|---|---|
| Release, 1962 | 6053 | 35.3 | 34.1–36.6 |
| Recovery, 1963 | 748 | 34.1 | 30.6–37.6 |
| Recovery, 1965 | 166 | 38.6 | 30.6–46.6 |

fishery on the Columbia River, and obtained an additional 4115 vertebrae from spawned-out fish on the Wenatchee River drainage. Among the 6165 fish, 156 fish had an adipose-maxillary clip. Of the clipped fish, 40.4% bore a tetracycline mark, a percentage not significantly different from that observed at time of release (Table V).

This field study showed that the presence of tetracycline in the sockeye salmon's skeletal system did not affect survival during either their 7-month residence in Lake Wenatchee or their 2-year stay at sea.

## GROWTH AND LENGTH–WEIGHT RELATION OF MARKED FISH

Several effects of tetracycline on growth are described in the literature. Large or prolonged doses of tetracycline administered during embryo formation tend to retard growth (Rolle and Bevelander, 1966; Saxén, 1965). Postnatal administration of tetracycline, however, often promotes growth (Guerrant, 1963). Bevelander and Goss (1962) demonstrated that regenerating scales of *Fundulus hetercolitus* given daily injections of TC had normal dimensions but contained less calcium. Similarly, Kobayashi et al. (1964) demonstrated that a single dose of tetracycline inhibited mineralization in goldfish scales. We have not noted growth promotion, retardation, or structural weakness in the bone tissue of salmon as a result of tetracycline administration.

As mentioned in the preceding section, seaward-migrating sockeye salmon from Lake Wenatchee were sampled, measured, weighed, and divided into four categories depending on the presence or absence of clips or tetracycline marks. Length–weight relation was calculated for fish of each category upon recovery. The method used in calculation is based on a length–weight relation as described by Rounsefell and Everhart (1953). Within the sample several hundred fish were not included because of incomplete measurements or the presence of 3-year-old sockeye of the 1960 year-class. The values of $c$ and $n$ in the equation $W = cL^n$, where L represents fork length in millimeters for fish 75–125 mm, and W weight in grams, are given in Table VI. The calculations show a decidedly lower constant and exponent for clipped fish as compared with fish that were unmarked or marked only with OTC. The small difference between the tetracycline-marked and unmarked fish demonstrates that administration of tetracycline did not impair growth.

TABLE VI. Length–weight relation ($W = cL^n$) of sockeye salmon recovered at time of migration out of Lake Wenatchee in 1963.

| Category | Number of fish | $c$ | $n$ |
|---|---|---|---|
| OTC and clip | 144 | $4.2676 \times 10^{-4}$ | 2.75495 |
| Clip only | 269 | $4.4397 \times 10^{-4}$ | 2.76369 |
| OTC only | 242 | $5.6968 \times 10^{-4}$ | 2.82608 |
| No mark | 710 | $5.9549 \times 10^{-4}$ | 2.83403 |

In our field study at Klaskanine Hatchery (designed to test permanence of a tetracycline mark) we recorded the fork length of each jack and adult coho salmon sampled upon return to the hatchery. A comparison of length frequencies of jack and adult returnees (Fig. 2) again indicated that tetracycline administration had no effect on growth.

## FACTORS IN COST OF MARKING WITH TETRACYCLINE

Several important factors affect the cost of marking with tetracycline. We fed 250–400 mg/kg body weight 3 or 4 consecutive days primarily to obtain 100% marking, and thus reduce one variable in analysis. Evidence from other animals (Dickinson et al., 1955) indicates that once a high level of antibiotic is attained, it can be sustained by lower subsequent doses. Gradual reduction in dosage of tetracycline may result in less than 100% marking, but costs could be cut one-third or more. Also, through use of low-calcium diets and potentiators, costs may be reduced even further by increasing absorption and thus lowering the dosage required to obtain a satisfactory mark.

Another cost consideration is related to the size of the fish at time of marking. Since the amount of tetracycline given is a function of body weight, it appears most economical to mark at the smallest possible size. The amount of fluorophore deposited, however, and hence the intensity of the mark, are partly dependent upon the amount of proliferating bone area available at time of marking. Again, subsequent bone growth and reabsorption are usually greatest in the area of bone origin. Inadequate intensity of the mark and subsequent overlying bone may render detection difficult and increase cost of analysis. To minimize both the cost of marking and the cost of detection, an optimum size for marking should be considered.

## SUMMARY

Our studies on Pacific salmon and rainbow trout have shown that oral administration of tetracycline antibiotics in adequate doses causes deposition of fluorescent material on the surface of the growing bone. The resulting mark is readily detectable by fluorescent microscopy and is apparently permanent, since no diminution of the mark occurred in fish recovered 3½ years after marking.

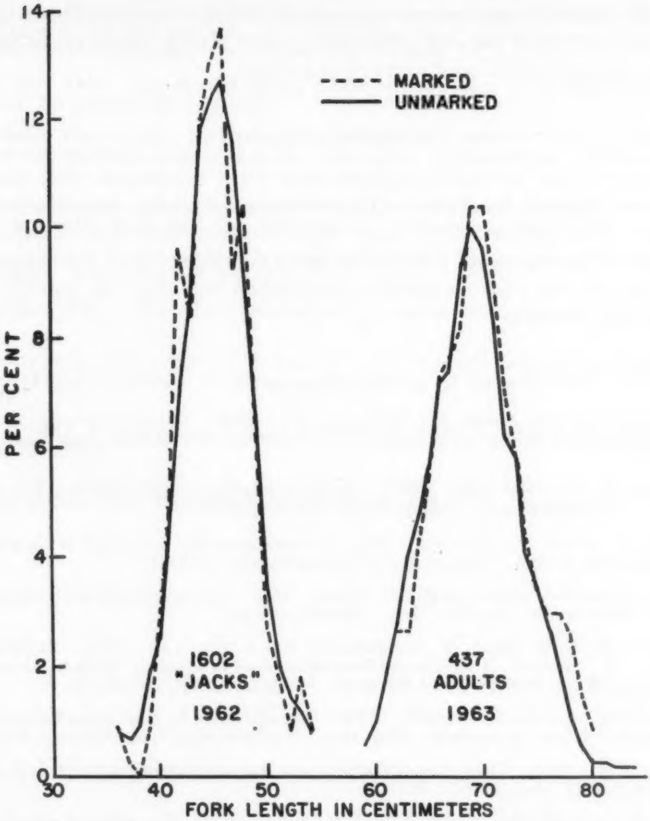

FIG. 2. Length frequencies, in per cent, of tetracycline-marked and unmarked jack and adult coho salmon that returned to Klaskanine Hatchery.

We found that the oral dosage levels necessary for deposition of a detectable mark may be as low as 25 mg per kilogram of body weight per day; but, the most satisfactory and consistent results were obtained by feeding a minimum of 250 mg/kg body weight daily for 3 or 4 consecutive days. There is evidence that absorption of antibiotics from the gut may be increased by using diets with low calcium content and a potentiator; thus the dosage level required for marking may be reduced.

No immediate mortalities from feeding tetracycline antibiotics have been experienced, and all evidence accumulated from field studies indicates no long-term adverse effect on growth or survival.

Marking fish with tetracycline antibiotics offers a number of advantages. Entire hatchery populations may be marked easily without individual handling.

The mark applied is permanent throughout the life of a fish. No mutilation or visible contrast with normal coloration occurs which might cause differential survival among marked and unmarked groups.

## ACKNOWLEDGMENTS

Many of our laboratory experiments were conducted with tetracycline antibiotics donated by Lederle Laboratories Division, American Cyananid Company, Pearl River, New York, and Charles Pfizer and Company, New York City. The success of our field studies depended upon the cooperation of personnel from the Fish Commission of Oregon and the U.S. Bureau of Sport Fisheries and Wildlife.

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# Histopathology of "Gas-Bubble" Disease in Salmon Fingerlings[1,2]

By Gilbert B. Pauley and Roy E. Nakatani

*Biology Department*
*Battelle Memorial Institute, Pacific Northwest Laboratory*
*Richland, Washington*

## ABSTRACT

Chinook salmon fingerlings were diagnosed as having "gas-bubble" disease. Gross symptoms were similar to those described by other investigators. The histopathology associated with this disease has not been previously reported. The following 10 tissues and organs were examined: gills, liver, spleen, heart, kidney, intestine, stomach, roof of the mouth, skin, and muscle. All exhibited histopathological changes with the exception of the hearts and stomachs, which did not differ from the control fish. The most striking pathological changes occurred in the roof of the mouth of all fish examined.

## INTRODUCTION

GAS-BUBBLE DISEASE was first observed in aquaria fish by Gorham (1898). This disease was also noted in aquaria fish by Marsh (1903), which led to a more detailed description of the cause, symptoms, and mechanics of gas-bubble disease (Marsh and Gorham, 1904).

Rucker and Hodgeboom (1953) briefly discussed the gas-bubble disease problem in the Puyallup trout hatchery. The gas-bubble disease problem and its probable causes at Cultus Lake trout hatchery were described by Harvey and Smith (1961). Rucker and Tuttle (1948) described a method of removing supersaturated nitrogen from hatchery water supplies. Westgard (1964) noted the problems associated with gas-bubble disease and methods for correcting the problems in the adult salmon spawning channel at McNary Dam. Alikunhi et al. (1951) found that carp fry, cultured under laboratory conditions, were extremely sensitive to gas-bubble disease, possibly due to excessive oxygen production. This disease is usually encountered in hatcheries, spawning channels, aquaria, or at low plunge basin water falls or dams, when water falling over the spillways becomes supersaturated. Special methods of controlling the amount of dissolved gases in the water are required to prevent excessive mortalities in these situations (Harvey and Smith, 1961; Westgard, 1964).

Although rare, some naturally occurring mortalities of fish due to gas-bubble disease have been recorded in black crappies, northern pike, walleyes, and cyprinids from Lake Kaubesa, Wisconsin (Woodbury, 1941); in trout from spring ponds of the River Bosna, Yugoslavia (Rukavina and Varenika, 1956);

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in large numbers of seatrout and in small numbers of several other marine fish from upper Galveston Bay, Texas (Renfro, 1963). Mortalities observed in the hatcheries, spawning channels, and aquaria are thought to have been due to supersaturation of nitrogen or oxygen caused by leaks in the hydraulic pipes or by heating cold water for temperature control. The mortalities observed in nature are thought to have been caused by extremely high concentrations of dissolved oxygen in the water produced by abnormally large algae blooms.

The literature pertaining to gas-bubble disease and its effects on fish has been reviewed by Bishai (1960). In a recent book dealing with diseases of lower vertebrates (Reichenbach-Klinke and Elkan, 1965), gas-bubble disease is only mentioned briefly.

Chinook salmon fingerlings, *Oncorhynchus tshawytscha*, that were dying in a public aquarium at Rocky Reach Dam, Washington, were diagnosed as having "gas-bubble" disease, with obvious large and numerous gas bubbles present on their bodies and heads. Most investigators that have encountered gas-bubble disease have described in detail the gross pathology associated with the diseased fish. However, there have been no previous records of the histopathology associated with this disease.

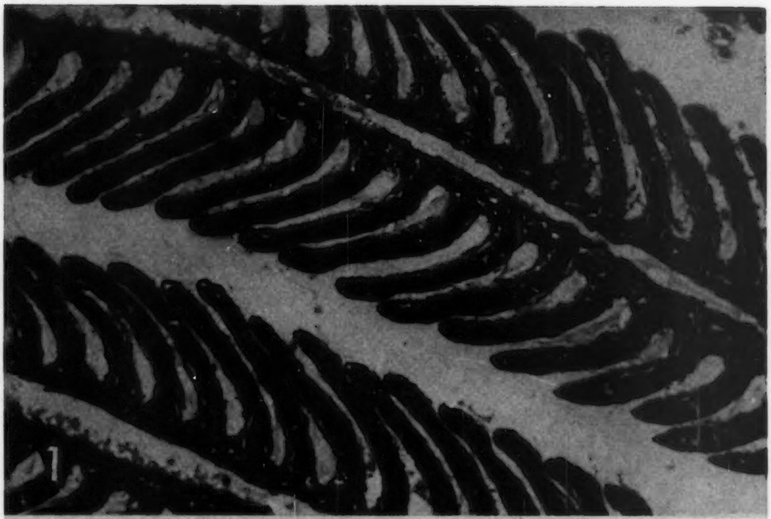
## MATERIALS AND METHODS

Several fish, approximately 6 inches long and 1 year old, that exhibited a light, but detectable, case of the disease were fixed for histopathological examination to determine if it affected specific organs or tissues in its early stages. Control fish of similar size and age were sampled from the hatchery at the Pacific Northwest Laboratory, because all fish in the Rocky Reach aquarium were afflicted with the disease. Samples of the following tissues and organs were preserved in methanol for histopathological examination: gills, liver, spleen, heart, kidney, intestine, stomach, roof of the mouth, skin, and muscle. Tissue sections were stained with Mayer's hematoxylin and eosin. Diseased and control tissues were fixed similarly and stained together to eliminate the occurrence of artifacts. The young fingerlings at the Rocky Reach Dam aquarium were originally obtained from L. R. Donaldson's stock at the University of Washington, Seattle.

## RESULTS

Gross examination of the fingerlings showed gas bubbles on the body, head, the peritoneal lining of the body cavity, and the oral cavity. The hearts and stomachs of the diseased fish appeared normal upon histological examination, not differing from the controls. However, all other tissues of the diseased fish exhibited definite histopathological changes.

The diseased fish possessed swollen, edematous gill filaments (Fig. 2, compare to Fig. 1), which showed hydropic degeneration of the lining squamous epithelium in many instances. Hemolyzed red blood cells were common in the gill filaments of all diseased fish.



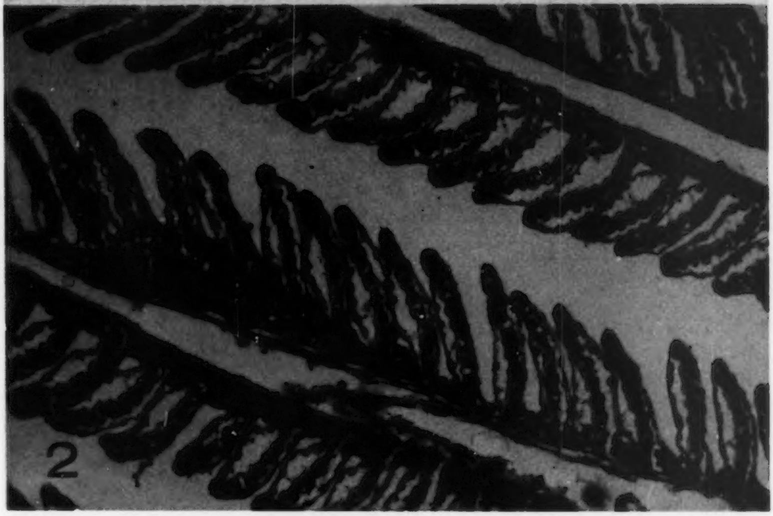
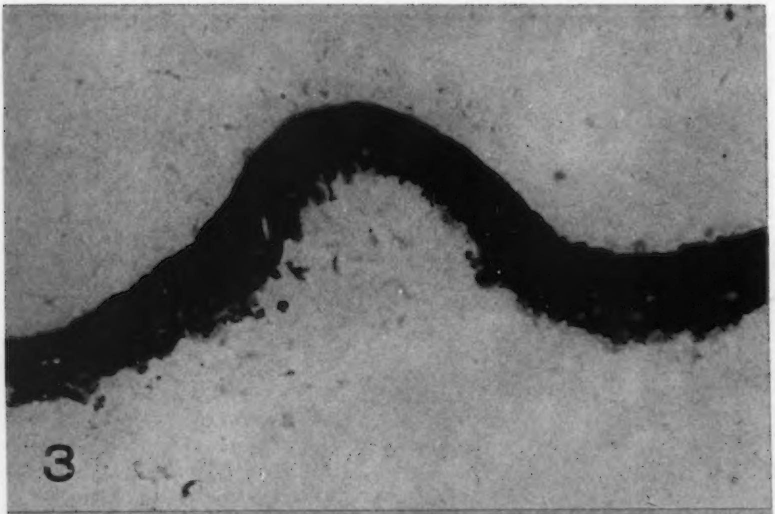


FIG 1. Normal gill of a salmon fingerling. 155×.

FIG. 2. Gill of salmon fingerling afflicted with gas-bubble disease. Note the swollen, edematous appearance of the individual filaments. 155×.



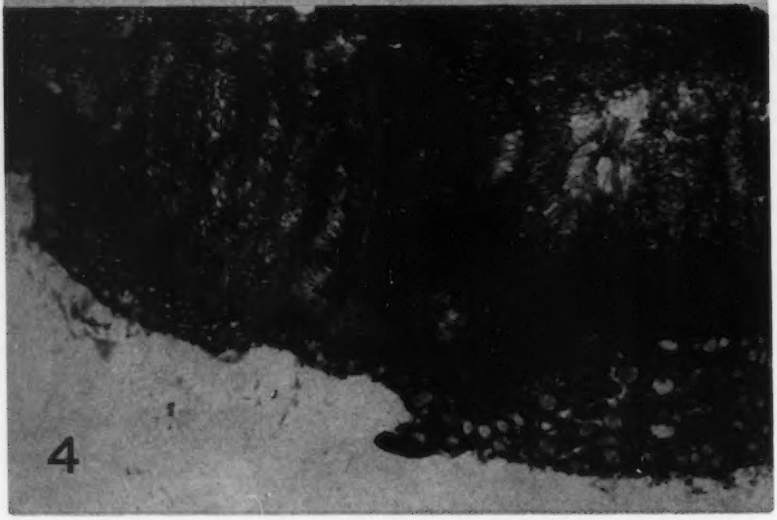


FIG. 3. Normal tissue from the roof of the mouth of a salmon fingerling. 155×.

FIG. 4. Tissue from the roof of the mouth of salmon fingerling possessing gas-bubble disease. Note the swollen appearance of both the epithelial and connective tissue layers. 155×.

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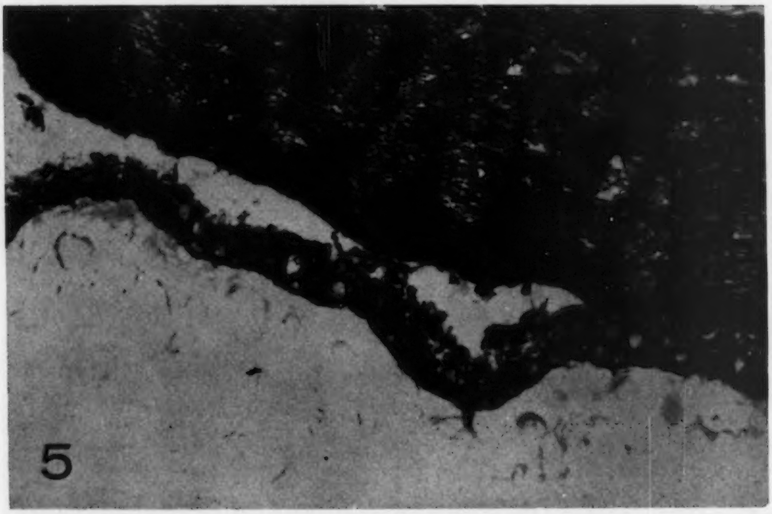

FIG. 5. Tissue from the roof of the mouth of salmon fingerling possessing gas-bubble disease. Note the swollen appearance of the tissue and the vesicles between the epithelium and connective tissue. 155×.

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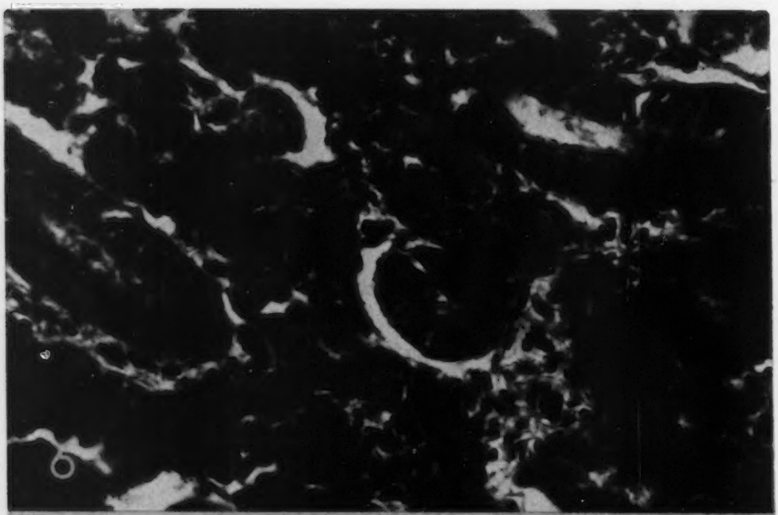


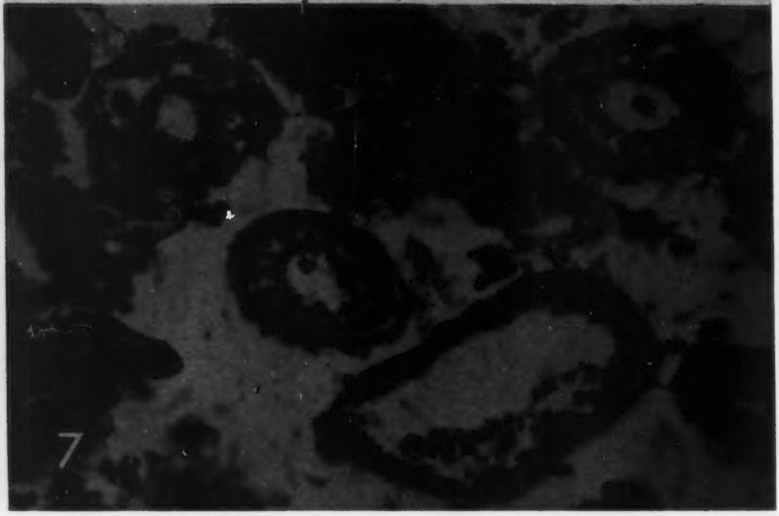
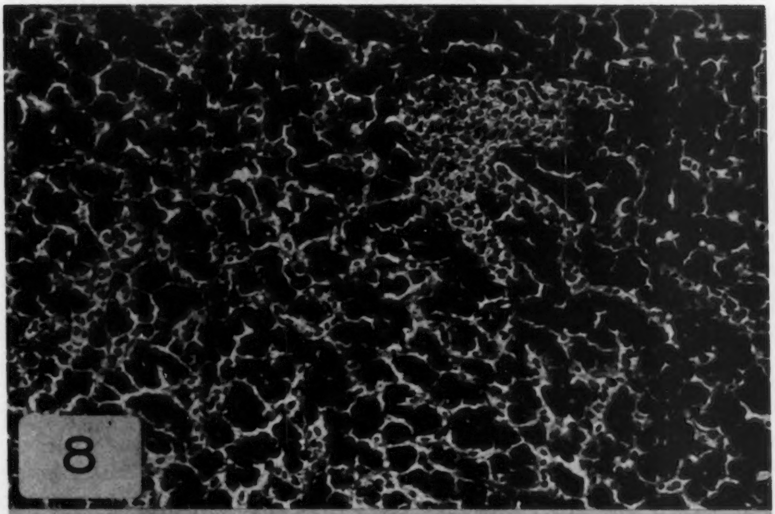


FIG. 6. Normal kidney from salmon fingerling. 390×.

FIG. 7. Kidney of salmon fingerling exhibiting gas-bubble disease. Note the pyknotic nuclei and lighty stained cytoplasm of the epithelial cells. 390×.



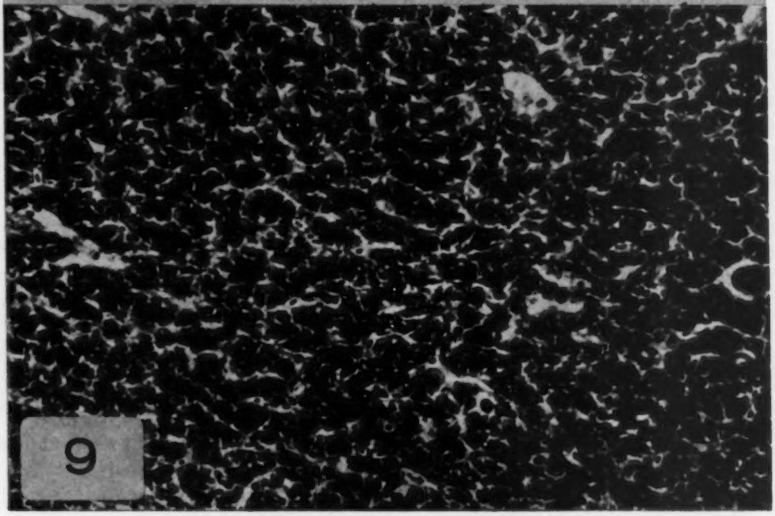


FIG. 8. Normal liver from a salmon fingerling. 155×.

FIG. 9. Liver from a salmon fingerling afflicted with gas-bubble disease. Note the lighty stained cytoplasm of the liver cells, the obscured normal architecture of the organ, and the almost complete lack of red blood cells. 155×.

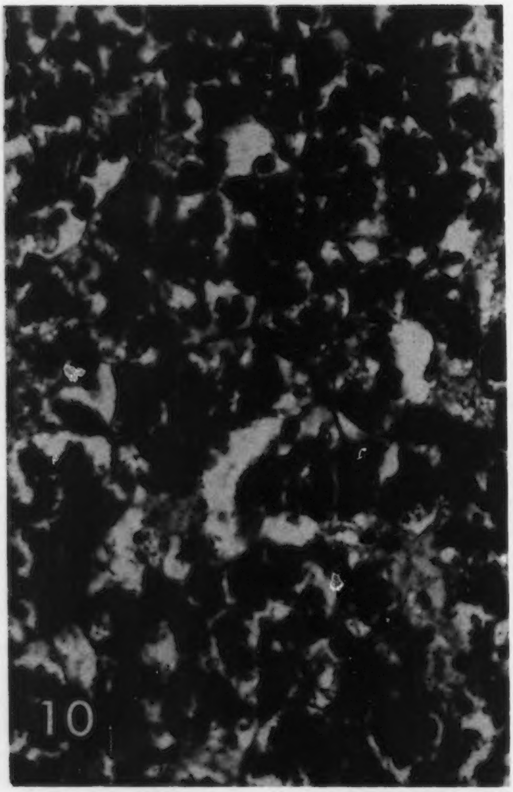


FIG. 10. A fingerling salmon afflicted with gas-bubble disease whose liver cells exhibit marked degenerative changes. Note the irregular staining cytoplasm and pyknotic nuclei. 390×.

The most striking pathological changes occurred in the roof of the mouth where the squamous epithelium had undergone hydropic degeneration and the dense connective tissue beneath it was edematous, greatly hypertrophied, and possessed karyolytic (faintly stained and lacking basophilism nuclei (Fig. 4 and 5, compare to Fig. 3). In some cases, large vesicles were present between the connective tissue and the epithelial layer, resulting in a separation of the epithelium from the underlying connective tissue (Fig. 5).

The spleens of all infected fish showed an apparent reduction of the white pulp and an abnormal hemolysis of red blood cells.

The diseased animals all possessed kidney tubules with epithelial cells exhibiting vesicular, lightly stained cytoplasm and pyknotic (shrunken and darkly staining) nuclei (Fig. 7, compare to Fig. 6). Many of the epithelial cells were beginning to slough away from the basement membranes. The kidneys had an increased number of hemolyzed red blood cells.

Necrosis was apparent in the intestines of the diseased fish where the epithelial cells possessed vesicular, lightly staining cytoplasm, faded nuclei, and in many cases the distal ends of these cells were ruptured. Beneath the intestinal epithelium, the granule cells of the granulosum possessed vesicular cytoplasm and lightly stained nuclei. In some fish this layer of cells was nonexistent.

Skin and muscle samples were taken as a single unit from the lateral line region of the fingerlings. The muscle fibers were atrophied, edematous, and separated from the sarcolemma. The muscle cells exhibited a light staining cytoplasm which had lost its characteristic striated appearance. The muscle nuclei were pyknotic and, in many instances, completely separated from the muscle fibers. In many respects, the epithelial layer of the skin showed pathologic changes similar to those observed in the epithelium of the roof of the mouth.

The liver cells had undergone degenerative changes, with vesicular, hypertrophied cytoplasm and pyknotic nuclei. In some instances, the liver cells were so enlarged that the normal architecture of the organ was obscured and the red blood cells normally present in the liver sinusoids were absent (Fig. 9, compare to Fig. 8).

## DISCUSSION

The gross pathology observed in the fingerling salmon from Rocky Reach Dam has been noted by other investigators. However, some additional symptoms of the disease have been reported by other workers, including swollen air bladders and gas bubbles in the gill arch mucosa, the intestine, the bulbus arteriosus of the heart, the eyes, and between the membranes connecting the fin rays. Microscopic examination of the gills has shown the branchial arterioles completely filled with gas bubbles (Renfro, 1963).

One puzzling aspect of the malady is the apparent recovery ability of fish in advanced stages of the disease (Rukavina and Varenika, 1956; Fujihara, personal communication, 1965). This is extremely perplexing upon observing the systemic destruction of cells and tissues by this disease in its early stages.

However, this apparent ability to recover is probably related to the supersaturation level of the water, the water temperature, the duration of exposure to the supersaturated water, and the size and species of the fish involved. Small fish appear to be highly susceptible to gas-bubble disease (Alikunhi et al., 1951; Harvey and Cooper, 1962). Westgard (1964) has discussed the physical and biological problems associated with gas-bubble disease and some possible solutions. However, the disease's etiology, including the stage of the disease at which desirable species of fish will not recover, has not been thoroughly studied.

The histopathological changes observed in the salmon fingerlings due to gas-bubble disease are similar to the pathological changes that have been observed in other fish due to chemical toxicity (Christie and Battle, 1963; Andrews et al., 1966), which include congested and edematous gill filaments, edematous muscle fibers, and degenerative liver changes with variations in staining intensity and distortion of the liver cells. The striking pathological changes that resulted in the "swollen" roof of the mouth appear to be unique to gas-bubble disease and we are using this as the diagnostic characteristic for detecting gas-bubble disease in its early stages. Death in fish suffering from gas-bubble disease is apparently caused by necrosis of vital organs and tissues, probably due to anoxia resulting from capillary occlusion by gas emboli.

## ACKNOWLEDGMENTS

The cooperative efforts contributed by Messrs D. E. Schoeneman, T. K. Meekin, A. C. Moser, and G. Tangen of the Washington State Department of Fisheries are gratefully acknowledged.

We are grateful to Dr William J. Clarke, Manager of the Pathology Section of the Pacific Northwest Laboratory, for his assistance and interpretation of many of the histopathological results and critical review of the manuscript. Collection of the samples was made by Mr P. A. Olson of the Pacific Northwest Laboratory. Technical assistance of Mr J. D. Maulsby is gratefully acknowledged.

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# Protein in Extracts of Prerigor and Postrigor Scallop Striated Muscle[1]

By J. J. Matsumoto,[2] W. J. Dyer, J. R. Dingle, and D. G. Ellis

*Fisheries Research Board of Canada*
*Halifax Laboratory, Halifax, N.S.*

## ABSTRACT

**The extraction of protein from the striated muscle (prerigor and postrigor) of scallop (*Placopecten magellanicus*) by salt solutions of various strengths and at *p*H 7 was investigated. With postrigor muscle the structural proteins appeared in the extract only at ionic concentrations ($\Gamma/2$) greater than 0.3 .With prerigor muscle, most of the protein was extracted at $\Gamma/2 = 0.15$. A large part of this protein precipitated from the extract upon standing, and when redissolved in stronger salt solutions resembled myosin B in sedimentation behavior and ultraviolet absorption.**

## INTRODUCTION

In contrast with the fishes and higher vertebrates, the muscle of some of the marine invertebrates is characterized by an abundance of a protein called, variously, tropomyosin A and paramyosin (Bailey, 1957). The role of this protein in the mechanism of adductor muscles has been the principal reason for recent interest in this field (see brief review by Riddiford and Scheraga, 1962). Various Japanese workers have investigated the nature of "myosin B" extracts from a scallop *Pecten yessoensis* and other marine animals (Yagi et al., 1954; Tonomura et al., 1955, 1956; Matsumiya et al., 1957; Maruyama and Matsumiya, 1957; Maruyama and Tonomura, 1957). Matsumoto (1957, 1958) observed the unusual extraction with water of a protein with contractile properties from the mantle muscle of squid. Differences in the ease of extraction of paramyosin from molluscs and from vertebrates (Laki, 1962) also indicate that the structure of the muscle of the lower marine animals may differ from that of mammals, upon which most investigations have been carried out.

The rise in importance of the scallop fishery on the Northwest Atlantic banks in recent years has stimulated an interest in the basic knowledge of the edible muscle of this mollusc *Placopecten magellanicus*. This paper deals with the extractability of the proteins of the striated portion of its adductor muscle.

## MATERIALS AND METHODS

### Scallops

For the prerigor experiments, live scallops obtained from both the inshore and offshore fisheries were held for up to 1 month in seawater aquaria at the

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[1] Received for publication September 14, 1966.

[2] Postdoctoral Fellow of the National Research Council of Canada. Present address: Sophia University, Tokyo, Japan.

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laboratory at 2–3 C. The average diameter of the shells was 12.2 ± 1.8 cm. Postrigor muscle was obtained from the local market. The adductor muscle consists of two parts: the white muscle, which is striated (Parker and Haswell, 1897), and the much smaller yellow part, composed of nonstriated tissue. Only the white part was used in these experiments. Proximate analysis showed that it consisted of 80% water, 12% protein, 0.5% lipid, and 1% ash. The remainder is accounted for by glycogen and other organic metabolites. Extraction of the muscle with 0.4 M KCl, followed by three extractions with 0.01 N NaOH, yielded 98–99% of the total muscle protein, leaving 1–2% as stroma.

### EXTRACTION

Samples of muscle (approximately 22.5 g) were homogenized with 19 volumes of extracting solution in a Waring blendor fitted with a baffle plate to prevent vortex formation (Dyer et al., 1950), and then centrifuged for 20 min at 1850 *g*. For "zero" ionic concentration, water was used; otherwise the extractant was 0.003 M $NaHCO_3$ or 0.05 M potassium phosphate buffer (*p*H 7.0), the total ionic concentration ($\Gamma/2$) being adjusted with KCl as required. Extractions were carried out in a cold laboratory at 2–3 C.

### DETERMINATION OF PROTEIN

Total nitrogen in muscle samples was determined by macro-Kjeldahl analysis. Nonprotein nitrogen was determined in the supernates of centrifuged extracts of muscle in 5% trichloracetic acid, using the micro-Kjeldahl procedure of Campbell and Hanna (1937). Protein nitrogen in the salt extracts was measured by the biuret procedure of Snow (1950). Correction was made for a turbidity in the samples (usually about 7% of the optical density) by the use of suitable blanks. "Actomyosin" was determined in the extracts by diluting them to $\Gamma/2 = 0.05$, centrifuging after standing overnight at 2 C, redissolving the precipitate in N/10 NaOH, and assaying the protein nitrogen by the biuret procedure.

### SEDIMENTATION, ELECTROPHORETIC, AND DRF MEASUREMENTS

Sedimentation runs were made in a Spinco model E ultracentrifuge at 59,780 rpm and rotor temperature 20 C. Apparent sedimentation constants were calculated and are given in Svedberg units, s, ($cm/sec/dyne/g \times 10^{13}$); no corrections for viscosity were made. Free-boundary electrophoresis runs were carried out in a Spinco model H apparatus, after dialysis of the sample for more than 18 hr against 40 volumes of buffer, changed once during the dialysis. Double refraction of flow (DRF) was estimated roughly by stirring the sample between crossed polaroid discs and judging the intensity of the cross.

## RESULTS

The results of extraction of postrigor scallop muscle at a series of ionic concentrations up to 1.5 are shown in Fig. 1. The material extracted up to $\Gamma/2 = 0.25$ presumably was composed of sarcoplasmic proteins, and amounted

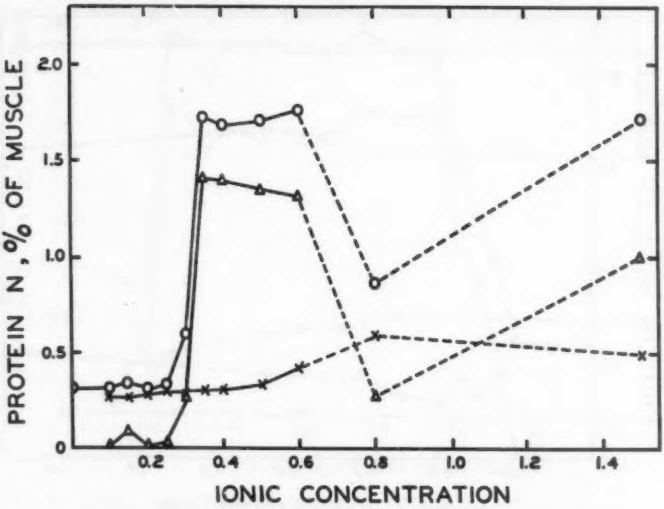

FIG. 1. Effect of ionic concentration on the extraction of protein from postrigor scallop muscle: ○, total protein extracted; △, actomyosin fraction; ×, "water-soluble" fraction (by difference).

to 20% of the total protein. A maximum of 99% of the total protein was reached at $\Gamma/2 = 0.35$–$0.40$, and at $\Gamma/2 = 1.5$. The character of the residue after centrifuging varied with the extracting solution. At $\Gamma/2 = 0$–$0.20$, and 0.35–0.60, the residue was well packed and the supernate readily separated. At $\Gamma/2 = 0.25$–$0.30$ the residue occupied the greater part of the mixture. At $\Gamma/2 = 0.80$, separation was very poor, and the low point on the graph represents the protein concentration in the thin clear supernatant layer. Separation improved again at higher salt concentrations. The actomyosin fraction is also shown in Fig. 1. Its maximum coincided with the maximum of extraction at $\Gamma/2 = 0.35$, and apart from the anomaly at 0.80, the amount of the fraction decreased gradually up to $\Gamma/2 = 1.5$.

These curves closely resembled those obtained for postrigor cod muscle (Fig. 2) (Fraser and Dyer, unpublished data) in which 20–25% of the total protein was extracted up to $\Gamma/2 = 0.25$, and a maximum extraction of 89% was reached at $\Gamma/2 = 0.35$–$0.40$. Anomalous behavior at intermediate concentrations was not observed in this case, however.

Whereas prerigor cod muscle yielded extracts similar in protein concentration to those from postrigor muscle (Fig. 2 and 3), extraction of prerigor scallop muscle presented some unexpected features. A very large yield (76% of the total protein) was already attained at $\Gamma/2 = 0.10$, with a maximum extraction of 92% at $\Gamma/2 = 0.30$ (Fig. 4). With such a large yield at $\Gamma/2 = 0.10$, a major part of the structural proteins must have been present in the

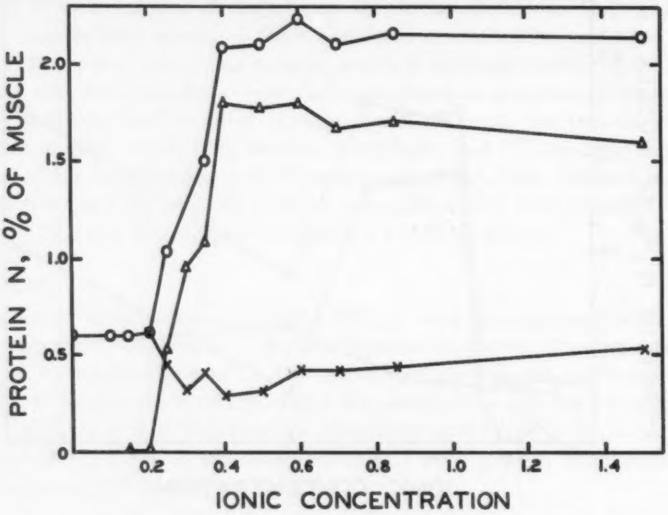

FIG. 2. Effect of ionic concentration on the extraction of protein from postrigor cod muscle: ○, total protein extracted; △, actomyosin fraction; ×, "water-soluble" fraction (by difference).

extract, even though these proteins from muscle of higher animals (e.g. cod, Dingle, 1958) are not normally soluble in salt solutions of this strength. The extract with water was not viscous, and did not show DRF, but those beyond $\Gamma/2 = 0.10$ showed marked DRF, and were viscous between 0.15–0.60. The residue in the aqueous extracts was voluminous; in extracts at $\Gamma/2 = 0.70$ and 0.80 there was a thin gel-like structure in the supernatant which exhibited thixotropic behavior. The residues at other concentrations were well packed.

Upon standing overnight at 0 C, a considerable amount of protein precipitated from some of the extracts. The concentrations of protein remaining soluble then followed a curve similar to that obtained in extraction of postrigor muscle.

From the appearance after centrifugation of the prerigor extracts made at $\Gamma/2 = 0.7$–$0.8$, and in view of their unstable nature, the prerigor and postrigor extracts made under these conditions seem to be similar. A slight difference in ease of sedimentation could account for the apparent differences in extractability suggested by the curves of Fig. 1 and 4. The reason for the low solubility of the proteins at this salt strength is, however, not known.

The nature of the prerigor extracts was investigated further by analytical ultracentrifugation. Sedimentation patterns of extracts of scallop muscle made at $\Gamma/2 = 0.4$ and 0.6 showed the presence of four components (arbitrarily numbered I–IV in order of descending sedimentation constant; Fig. 5C, D). These patterns were similar to those previously found for extracts of cod muscle

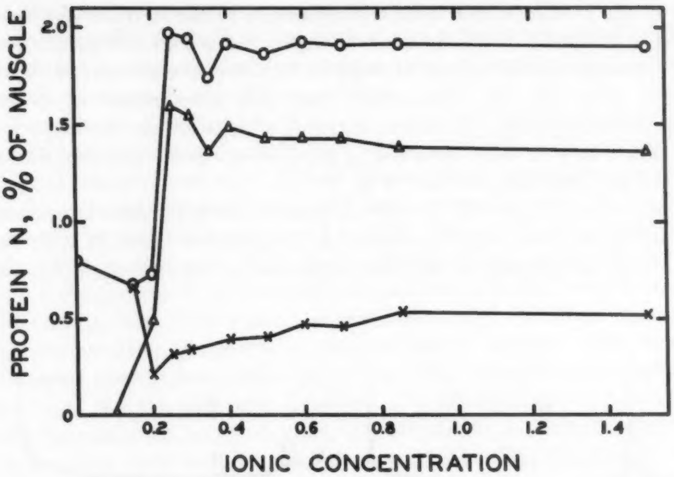

FIG. 3. Effect of ionic concentration on the extraction of protein from prerigor cod muscle: ○, total protein extracted; △, actomyosin fraction; ×, "water-soluble" fraction (by difference).

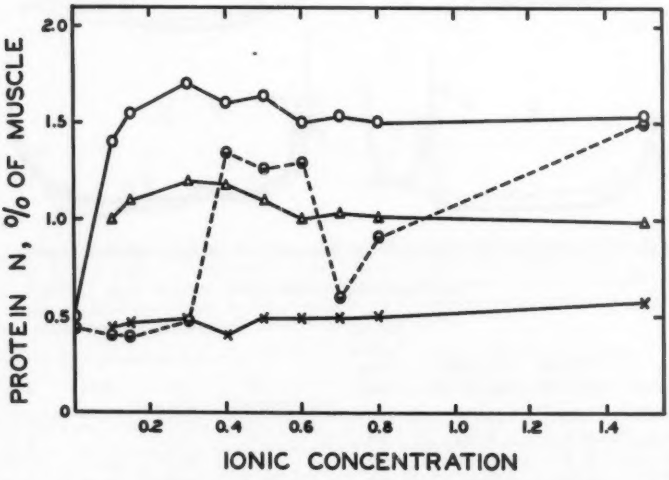

FIG 4. Effect of ionic concentration on the extraction of protein from prerigor scallop muscle: ○, total protein extracted; △, actomyosin fraction; ×, "water-soluble" fraction (by difference); ◒, proteins remaining in the supernatant fluid after removal of the spontaneous precipitate formed upon standing at 0 C.

(Dingle et al., 1963). As the ionic concentration of the extracting solution was increased to 0.8 and 1.5, the amounts of components I and II progressively decreased, accompanied by a large increase in III, while the amount of IV remained unchanged (Fig. 5E, F). This effect had also been noted in cod extracts (Ellis, unpublished data). In water extracts of scallop, on the other hand, no components I and II were detected; a small sharp peak possibly due to component III was the main feature (Fig. 5A).

In an extract made at Γ/2 = 0.15, despite the high protein concentration of 0.72 mg N/ml, only small amounts of components I and II were detected, together with heterogeneous material sedimenting very slowly (Fig. 5B). The

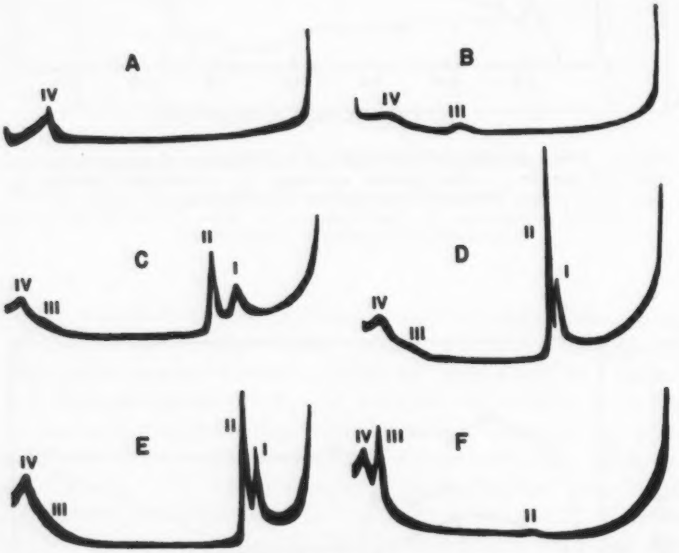

FIG. 5. Sedimentation patterns of extracts of prerigor scallop muscle. Sedimentation to the right.

| Pattern | Γ/2 of extract | Protein (*mg N/ml*) | Centri-fuging time (*min*) | Sedimentation constants I | II | III | IV |
|---|---|---|---|---|---|---|---|
| A | 0.0 | 0.30 | 39 | – | – | – | 4.3 |
| B | 0.15 | 0.49 | 39 | – | 36.7 | 10.2 | 3.7 |
| C | 0.40 | 0.60 | 28 | 31.8 | 27.9 | 6.6 | 3.2 |
| D | 0.60 | 0.60 | 28 | 27.0 | 25.5 | 7.0 | 3.2 |
| E | 0.80 | 0.67 | 23 | 41.7 | 39.2 | 5.9 | 2.7 |
| F | 1.5 | 0.71 | 23 | 78.4 | – | 4.9 | 2.5 |

amounts of these components were too small to account for the total protein present. Presumably, the bulk of the protein was present as suspended particles not sedimented by the ordinary centrifuge used in preparation (1800 *g*) but very rapidly removed by the ultracentrifuge.

To investigate this further, part of a prerigor scallop muscle extract, made at Γ/2 = 0.15, was brought to Γ/2 = 0.40 with KCl, reblended, and recentrifuged; another part was allowed to precipitate spontaneously overnight at 0 C. The precipitate was separated, and reblended with KCl–phosphate (Γ/2 = 0.40), whereupon it dispersed readily. Part of the supernate was also brought to Γ/2 = 0.40 with KCl. The sedimentation pattern of the original extract (0.6 mg protein N/ml) showed components III and IV with traces only of I and II (Fig. 6A). The extract reblended at Γ/2 = 0.40, with the same protein concentration, showed a typical myosin B pattern with components I and II present in the normal amounts (Fig. 6B). This indicated that much of the structural protein was now dispersed. No precipitation or change in the ultracentrifuge pattern occurred when this solution was allowed to stand overnight, suggesting that the extract was now stable. The precipitate obtained

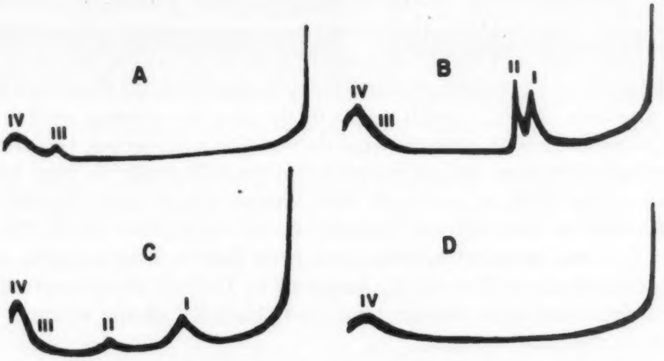

FIG. 6. Sedimentation patterns of an extract of prerigor scallop muscle made at Γ/2 = 0.15. A, untreated extract; B, same as A, but reblended at Γ/2 = 0.40; C, spontaneous precipitate from A, redispersed at Γ/2 = 0.40; D, supernatant fluid from A, after removal of the spontaneous precipitate. Sedimentation to the right.

| Pattern | Protein (*mg N/ml*) | Centrifuging time (*min*) | Sedimentation constants I | II | III | IV |
|---|---|---|---|---|---|---|
| A | 0.45 | 21.5 | 71.2 | 36.2 | 9.7 | 3.1 |
| B | 0.45 | 21.5 | 35.2 | 34.1 | 6.6 | 3.5 |
| C | 0.63 | 12.8 | 63.6 | 36.7 | 6.6 | 3.2 |
| D | 0.26 | 37 | – | – | – | 3.1 |

from the original extract upon standing, and redispersed as above, showed a characteristic myosin B pattern of a large component II with the usual accompanying III and IV (Fig. 6C). The supernate, on the other hand, was similar to the original extract in showing only components III and IV (Fig. 6D). This picture was unchanged when the supernate was made up to $\Gamma/2 = 0.40$. When compared by electrophoresis at $\Gamma/2 = 0.40$, the redissolved spontaneous precipitate and actomyosin thrice-precipitated from the $\Gamma/2 = 0.40$ extract also appeared to be similar (Fig. 7A, B).

The protein that spontaneously precipitated from extracts of prerigor scallop muscle made at $\Gamma/2 = 0.15$ therefore had a sedimentation and electrophoretic behavior very much like that of the proteins extractable from cod muscle only at ionic concentrations greater than about 0.4. Moreover, ultraviolet absorption spectra of the redissolved precipitates were typical of actomyosin, with absorption due to both tyrosine and tryptophan being evident. The protein was therefore not similar to the paramyosin reported by Bailey (1957) to exist in large amounts in smooth adductor muscle of Mollusca, and which resembled vertebrate tropomyosin in having no tryptophan.

In some ultracentrifuge runs a very heavy heterogeneous component was detected as a small, fast, rapidly spreading peak. Electrophoresis runs on the stable portion of $\Gamma/2 = 0.15$ extracts at *p*H 7.4 showed, in addition to several peaks attributable to the sarcoplasmic components, a large, very slow-moving peak (peak 6, Fig. 7C, D), the area of which could not be accounted for by the amount of protein present. These scallop muscles contained glycogen in large amounts (4–5%), and it seems likely that the unusual peaks observed in these two methods of analysis were due to this component. Under the mild conditions of extraction, the glycogen would probably exist as very large molecules, or as particles of relatively low charge (Orrell and Bueding, 1964).

The effect of postmortem changes on the extraction of scallop muscle at $\Gamma/2 = 0.15$ was followed by extracting from time to time samples of muscle stored in polyethylene film at 3 C. As shown in Table I, the amount of protein extracted decreased with storage time, and the DRF of the extracts also decreased with the onset of rigor.

TABLE I. Effect of postmortem aging on the extraction of scallop muscle at $\Gamma/2 = 0.15$. (Protein concentrations are given as grams N per 100 g wet muscle.)

| | | Fresh extract | | | | Extract aged 18 hr | | |
|---|---|---|---|---|---|---|---|---|
| Storage time (*days*) | *p*H of muscle | *p*H | DRF | Total protein | Acto-myosin | *p*H | DRF | Soluble protein |
| 0 | 7.3–7.6 | 7.2 | ++ | 1.41 | 0.95 | 7.1 | – | 0.53 |
| 1 | – | 7.0 | ++ | 1.41 | 0.98 | 6.6 | – | 0.43 |
| 4 | – | 6.9 | + | 1.21 | 0.91 | 6.5 | – | 0.33 |
| 8 | 5.7–6.0 | 6.7 | – | 0.39 | 0.14 | 6.4 | – | 0.29 |

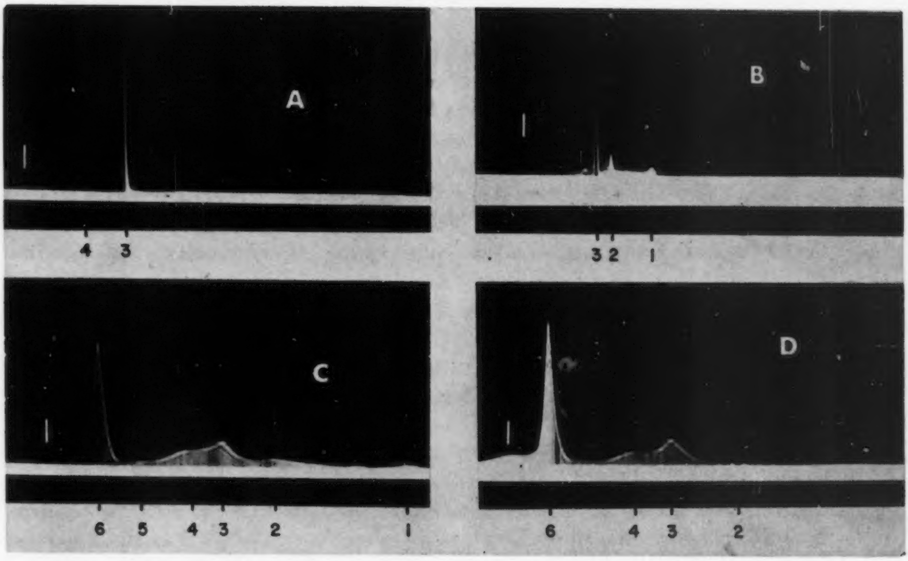

FIG. 7. Electrophoresis patterns of prerigor scallop muscle extracts. A, protein precipitating spontaneously from an extract at $\Gamma/2 = 0.15$, redissolved at $\Gamma/2 = 0.40$, *p*H 7.3; protein 0.50 mg N/ml; ascending limb, after 329 min at 2.07 v/cm. B, actomyosin thrice-precipitated from an extract made at $\Gamma/2 = 0.40$; protein, 0.35 mg N/ml; ascending limb, after 300 min at 2.05 v/cm. C, stable components in an extract at $\Gamma/2 = 0.15$; protein 0.26 mg N/ml; ascending limb, after 305 min at 4.46 v/cm. D, same as C, descending limb. Migration to the right in all cases.

The vertical white bar indicates the starting position.

Mobilities ( $\times -10^5$ cm$^2$ volt$^{-1}$ sec$^{-1}$)

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | – | – | 3.83 | 2.8 | – | – |
| B | 5.7 | 3.8 | 3.08 | – | – | – |
| C | 7.4 | 4.54 | 3.43 | 2.63 | 1.80 | 0.85 |
| D | – | 4.3 | 3.31 | 2.52 | – | 0.69 |

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## DISCUSSION

The occurrence of the structural proteins of scallop muscle in prerigor extracts made at low ionic concentration recalls previous experience with aqueous extracts of squid muscle (Matsumoto, 1957, 1958). Such extracts contained protein that could be precipitated by raising the ionic concentration to 0.05 by addition of KCl, and which resembled normal actomyosin according to its subsequent solubility behavior, the sensitivity of viscosity to adenosine triphosphate (ATP), and its ATPase properties. In the case of cod, Connell (1958) reported the occurrence of an actomyosin-like protein in extracts of prerigor muscle made at $\Gamma/2 = 0.10$ and 0.20 but not in extracts made with 0.05 M buffer only. In these two instances, however, the amount of protein extracted was relatively small (squid, 1–9% of total protein; cod about 21%), whereas with scallop, virtually complete extraction was attained at $\Gamma/2 = 0.15$. There was no indication that the "extra" squid protein precipitated upon standing, but the cod extract was found to gel after storage at 0 C, and in this respect resembled the scallop extracts. Mueller (1960) has demonstrated that in the presence of a resuspended microsomal fraction of rabbit muscle extract ("relaxing factor"), ATP, instead of causing the usual synaeresis of actomyosin gel in 0.05 M KCl, actually causes the solubilization of the proteins. This appears to be the most likely explanation of the phenomenon found in extraction of prerigor scallop and cod muscle with weak salt solutions. The behaviour of the scallop extracts in the ultracentrifuge suggests that the structural proteins were present as a dispersion of particles, rather than in true solution. The subsequent precipitation or gelling of the protein in these extracts is presumably due to the loss of the indigenous muscle ATP as a result of the ATPase activity of the structural proteins themselves. The failure to extract these proteins under similar conditions from postrigor muscle would be expected since little or no natural ATP would be present. On the other hand, there does not seem to have been any report of an analogous extraction of structural proteins from mammalian muscle at low ionic concentrations. Electron microscopic examination (Hanson and Lowry, 1960) does not suggest any fundamental difference in structure of muscles of higher and lower animals, and the reasons for the differences in the extractability of their proteins remain unknown.

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# NOTES

## A Plankton Sampler for Oyster Larvae

To predict the time and intensity of oyster spatfalls in Pendrell Sound, B.C., estimates of larval abundance are required. Because of the hydrographic situation 95% of the oyster larvae occur between the surface and a depth of 9 ft (2–7 m). Also the horizontal distribution of larvae in the area is not random and there is evidence of diurnal vertical movements.

A plankton sampler was therefore required to sample oyster larvae uniformly in a vertical column of water and to be used either as a spot or as a moving sampler.

The instrument was designed according to hydraulic theory in which equations and calculations were developed on: 1. Aperture head loss; 2. Pipe friction head loss; 3. Vertical variation in sampling rate; 4. Static pressure; 5. Effects of boat velocity on sampling rate; 6. Drag and life; 7. Mechanical strength.

The sampler (Fig. 1 and 2) is little more than a vertical length of perforated 2.5-inch (64-mm) anodized aluminum pipe, capped at its lower end and connected to a filter or plankton net, water meter, and suction pump at its upper end. The perforations are square-edged $\frac{3}{16}$-inch (4.8-mm) diam holes at 2-inch (51-mm) centres in the lower 8 ft (2.4 m) of the forward face of the pipe. The pipe size was chosen to give a head loss along its length very much less than the head loss at an aperture so that the variation in suction head available at the holes is small. A small splitter vane of 2 × 0.25-inch (51 × 8.3-mm) aluminum strap, welded to the rear face of the pipe, reduces drag and a more distant vane orients the pipe in the proper direction. To secure the sample a self-priming 1.25-inch (33-mm) centrifugal pump was used to carry the water through a Leeds-type water meter and into a plankton net.

In essence the device samples at a relatively large number of closely spaced discrete depths to provide an integrated sample. The difference in sampling rates between the top and bottom apertures is 1% but varies slightly with the speed of the boat and the rate of pumping. For moving samples the speed of the vessel was about 5 knots and pumping rate about 2 ft$^3$/min (57 liters).

Tests against one-level samplers showed it to be a satisfactory method for sampling oyster larvae in the area in question. It gave a slightly higher measure of larval abundance than the other sampling method and appeared to be less dependent on the time of sampling. A moving sample with the device, which results in only a single sample, provided as good an estimate of larval

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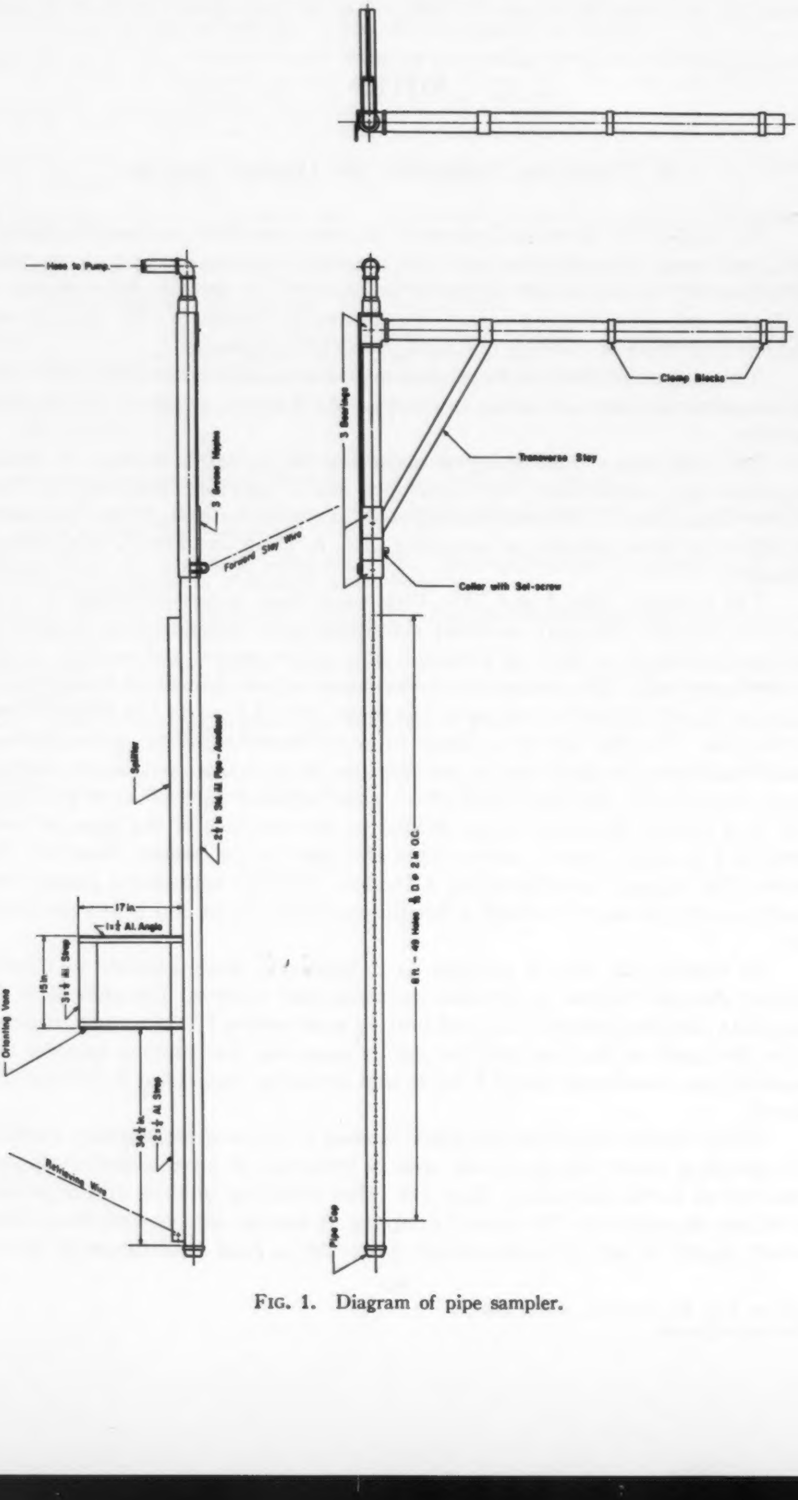
Hose to Pump.
3 Grease Nipples
Forward Stay Wire
3 Bearings
Clamp Blocks
Transverse Stay
Collar with Set-screw
8 ft - 49 Holes 3/8 D @ 2 in OC
Splitter
2½ in 3/16 Al Pipe - Anodized
17 in
1×½ Al. Angle
15 in
3×¼ Al Strap
Orienting Vane
34½ in
2×¼ Al Strap
Retrieving Wire
Pipe Cap

FIG. 1. Diagram of pipe sampler.

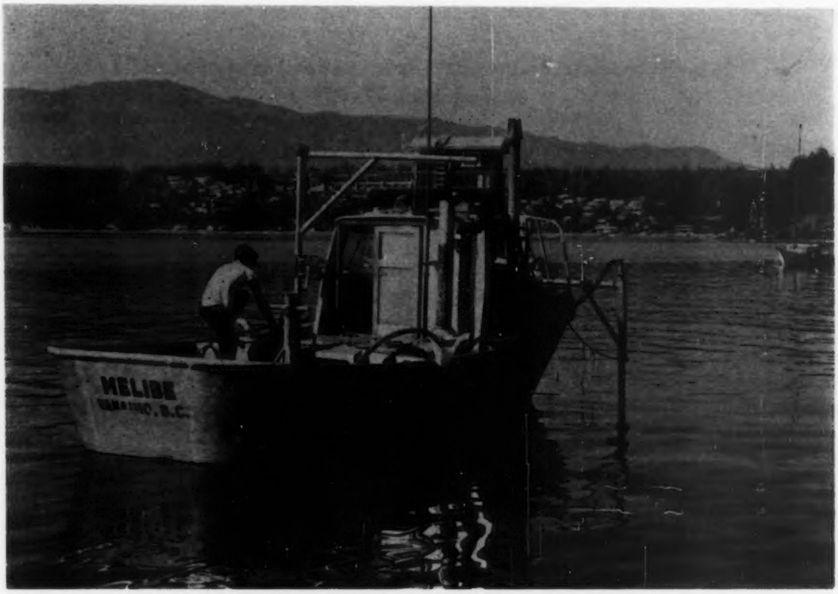

FIG. 2. Pipe sampler mounted on a vessel.

abundance as either the stationary one-level or pipe samples at several stations.

The hydraulic theory and sampling results have been reported in Biological Manuscript Report No. 887 which is available from the Biological Station, Nanaimo, B.C.

Fisheries Research Board of Canada
Biological Station, Nanaimo, B. C.

D. B. QUAYLE
L. D. B. TERHUNE

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# Phenotypic Characteristics of Rainbow Trout in the Great Lakes

Rainbow trout, *Salmo gairdneri* Richardson, were introduced into Lake Superior in 1895 and by 1905 they were firmly established. This species is now widely distributed in the Great Lakes watershed (MacKay, 1963). Most adult rainbow trout live in the lakes and spawn in tributary streams. Reproductive homing in this species is well documented (Lindsey et al., 1959). Homing (Hubbs, 1961) provides the initial step in genetic differentiation through reproductive isolation. Variations in environmental conditions, particularly temperature during early stages of development, tend to influence phenotypic features (Hubbs, 1922; Smith, 1957). In this report, morphological features of populations of rainbow trout from four geographically distinct Great Lakes areas (Fig. 1) are compared and found to show such differences.

*Methods.*—Samples of adult and immature wild rainbow trout were collected during the period 1962–64 inclusive from each study area with a back-pack fish shocker. Each fish was X-rayed and the data from the X-rays are presented in Tables I and III. The methods of measuring essentially follow the standards of Hubbs and Lagler (1958). A sample of 40 rainbow trout consisting of 10

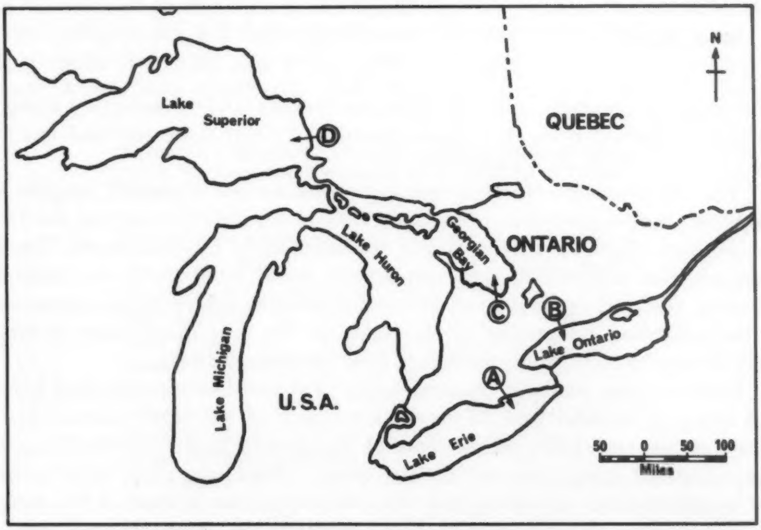

FIG. 1. Locations of sampling areas in four Great Lakes watersheds: A — North Creek (42°58′N, 80°37′W); B — Wilmot Creek (43°57′N, 78°37′W); C — Nottawasaga River (44°48′N, 80°07′W); D — Speckled Trout Creek (47°25′N, 84°34′W). The direction of drainage is indicated by arrows.

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TABLE I. Highly significant $(HS)_{.005}$ and significant $(S)_{.01}$ components of the analyses of variance of morphometric data. The ratios of the measurements to the standard lengths (sl)[a] were compared in each analysis. The sex and maturity components were highly significant in all cases where one was greater than another.

| Ratio | Component of analysis of variance | | |
|---|---|---|---|
| | Watershed | Maturity | Sex |
| Head length/sl | HS | Immature>Mature | Male>Female |
| Length of upper jaw/sl | HS | – | Male>Female |
| Snout length/sl | HS | Mature>Immature | Male>Female |
| Interorbital bony width/sl | – | Mature>Immature | – |
| Pelvic origin to tip of snout[a]/sl | S | Immature>Mature | Male>Female |
| Dorsal origin to tip of snout[a]/sl | – | – | Male>Female |
| Pelvic fin length/sl | HS | – | – |
| Dorsal fin length/sl | HS | Immature>Mature | – |
| Dorsal origin to pelvic origin[a]/sl | HS | – | – |
| Dorsal fin depressed length/sl | S | Immature>Mature | Male>Female |
| Dorsal fin height/sl | – | Immature>Mature | Male>Female |
| Anal fin length/sl | S | – | – |
| Anal fin height/sl | HS | Immature>Mature | – |
| Pectoral fin height/sl | – | Immature>Mature | Male>Female |
| Caudal peduncle depth/sl | HS | Immature>Mature | – |
| Anal origin to caudal base[a]/sl | – | – | – |
| Caudal peduncle length[a]/sl | – | Immature>Mature | – |

[a]From X-rays.

adult females, 10 adult males, 10 immature females, and 10 immature males was randomly selected from the approximately 75 trout collected and examined from each of the four watersheds.

The ratios of the various measurements to the standard lengths were compared using the analysis of variance (Snedecor, 1962) for each of the 17 sets of ratio data (Table I) after a probit transformation was employed. The total variation was partitioned into component parts attributable to watershed, maturity, sex, and random error. When a significant difference was encountered in the watershed component of the analysis, the four means were compared using Duncan's New Multiple Range Test (Snedecor, 1962).

Data on nine meristic characteristics (Table III) were compiled from 50 wild trout, 25 females and 25 males, from each of the four watersheds. The defined counts essentially follow those of Hubbs and Lagler (1958). These data were compared using the analysis of variance (Snedecor, 1962) after a square root transformation was employed. The total variation in each of the analyses was partitioned into component parts attributable to study area, sex, and random error.

Eggs from spawning adults from each of the four watersheds were incubated in the laboratory and reared to the yearling stage in water of uniform temperature, dissolved oxygen, and chemical composition. Three meristic characteristics (Table III) were enumerated in a sample of 50 cleared and stained (Hollister, 1934) cultured progeny from each watershed. Comparison of these data followed the same procedure as that of the wild samples but with no consideration given to the sex component of the sample.

*Observations and discussion.*—For the morphometric ratios, differences between sex and between maturity portions of the samples (Table I) are evident. Hartman (MS, 1954) found sharp inflections at sexual maturity in his log–log plots of body parts to fork length of rainbow trout. In both studies, the growth of the snout length and interorbital width relative to the body length increased following sexual maturity, whereas no difference was noted in the ratio snout to dorsal origin/body length.

Differences between watersheds (Tables I, II) were noted in 11 of the 17 morphometric ratios. Martin (1949), in experiments with rainbow trout reared under different diets and temperatures, found differences in body form between the experimental lots. Higher rearing temperatures increased certain ratios. In addition, a deficiency in diet increased some morphometric ratios. Different rearing temperatures and diets in the four Great Lakes watersheds could account for the variations in proportions of body parts.

In the meristic studies, the numbers of pelvic and caudal fin rays were found to be 10 and 19 respectively, in all of the 300 fish examined from the four watersheds. No significant differences between watersheds or between sexes were found in the nine comparisons (Table III) of meristic data. The cultured samples did not differ significantly between watersheds or from the wild samples. Needham and Gard (1959) in describing nine populations of rainbow trout from California and Mexico found the vertebrae ranged

TABLE II. Morphometric ratios found significantly different in the watershed component of the analyses of variance (Table I). The means found significantly greater $(>)_{.01}$ are indicated.

| Ratio | Watershed[a] |
|---|---|
| Head length/sl | D>A>B,C |
| Snout length/sl | A>B,C |
| Length of upper jaw/sl | D>B,C |
| Pelvic to snout/sl | A>D |
| Dorsal to pelvic/sl | A,B,C>D |
| Dorsal length/sl | B>A,D |
| Dorsal depressed length/sl | A>C,D |
| Pelvic length/sl | B>D>C>A |
| Anal length/sl | C>D |
| Anal height/sl | C,D>A,B |
| Caudal peduncle depth/sl | A,B>C>D |

[a] A — North Creek; B — Wilmot Creek; C — Nottawasaga River; D — Speckled Trout Creek.

TABLE III. Means and ranges (in parentheses) for meristic characters of the wild (W) and cultured (C) fish from each of the four watersheds.

| | Watershed | | | |
|---|---|---|---|---|
| Character | Wilmot Creek | North Creek | Nottawasaga River | Speckled Trout Creek |
| Vertebrae (W)[a] | 63.38<br>(61–66) | 62.86<br>(61–65) | 63.06<br>(61–65) | 62.74<br>(60–64) |
| (C)[b] | 62.76<br>(61–65) | 63.14<br>(61–64) | 62.70<br>(61–64) | 62.92<br>(62–64) |
| Dorsal fin rays (W)[a] | 14.92<br>(14–16) | 14.82<br>(13–16) | 14.80<br>(13–16) | 14.70<br>(13–16) |
| (C)[b] | 14.98<br>(14–16) | 14.76<br>(14–16) | 14.90<br>(14–16) | 15.04<br>(14–16) |
| Anal fin rays (W)[a] | 14.04<br>(13–15) | 13.98<br>(13–16) | 13.94<br>(12–15) | 14.12<br>(13–15) |
| (C)[b] | 14.22<br>(13–15) | 14.36<br>(13–15) | 14.26<br>(13–15) | 14.16<br>(13–16) |
| Scales along lateral line (W) | 122.56<br>(106–128) | 127.26<br>(119–136) | 124.64<br>(117–131) | 125.10<br>(118–129) |
| Scales above lateral line (W) | 24.66<br>(18–32) | 27.66<br>(26–31) | 27.16<br>(25–32) | 26.14<br>(20–32) |
| Scales below lateral line (W) | 21.06<br>(19–24) | 22.18<br>(21–26) | 21.18<br>(18–25) | 22.02<br>(18–24) |
| Branchiostegal rays (W) | 21.22<br>(18–24) | 22.22<br>(21–24) | 22.10<br>(20–24) | 22.12<br>(20–25) |
| Pectoral fin rays (W) | 13.68<br>(12–16) | 14.30<br>(13–15) | 13.94<br>(11–17) | 13.80<br>(12–15) |
| Gill rakers (W) | 17.90<br>(16–20) | 18.72<br>(17–21) | 18.90<br>(17–21) | 19.42<br>(18–21) |

[a] From X-rays.
[b] From cleared and stained samples.

from 57 to 66 with the greatest difference between means being 6.6 vertebrae. Great Lakes samples ranged from 60 to 66 with the greatest difference between means being only 0.64 vertebrae. This range falls within the upper limits of that of the west coast samples. The number of scales along the lateral line ranged from 105 to 126 in the west coast samples and from 106 to 136 in the Great Lakes samples. Needham and Gard (1959) found dorsal and anal fin rays varied from 8 to 12 and from 9 to 11 respectively, but Great Lakes samples varied from 13 to 16 and from 12 to 16 respectively. The discrepancies in these findings may be due to the fact that rudimentary rays, enumerated from X-rays, were included in the Great Lakes study.

The numbers of vertebrae and dorsal and anal fin rays are under genetic control but are affected by temperature and oxygen during the egg incubation period (Garside, 1966; Needham and Gard, 1959). These counts did not differ significantly between watersheds for the wild samples or the cultured samples, or for both. Incubation water temperatures in the laboratory ranged from 10.8 to 12.0 C and water temperatures in the four watersheds when spawning adults were captured varied from 8.5 to 13.0 C. It is assumed the water was saturated with oxygen in the watersheds as it was in the laboratory. In view of the similarity of temperature and oxygen levels in the field and in the laboratory, it appears that genetic control of these meristic features in the rainbow trout from the four watersheds was similar.

Only limited morphometric differences were noted in the four samples of rainbow trout from widely separated Great Lakes watersheds. Morphometric differences were present in some cases in the sex and maturity components of the samples. Meristic characters did not differ significantly between wild samples from the four watersheds or between cultured samples reared under controlled conditions from eggs of spawning adults in the four watersheds, or between totals for the various watersheds.

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University of Guelph
Guelph, Ont.

B. F. Bidgood[1]
A. H. Berst[2]

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[1] Present address: Alberta Dept. of Lands and Forests, Edmonton, Alta.
[2] Present address: Ontario Dept. of Lands and Forests, Maple, Ont.

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# *Triaenophorus crassus* Plerocercoids in Sockeye Salmon Smolts from the Kvichak River System, Alaska

*Triaenophorus crassus* Forel, 1868 (Cestoda: Pseudophyllidea) has been used as a "biological tag" to determine the ocean distribution of sockeye salmon, *Oncorhynchus nerka* (Walbaum), originating in Alaskan watersheds draining into the southeastern Bering Sea (Margolis, 1963). The incidence of this parasite in samples of smolts and adults taken in the years 1955 through 1959 from various river systems of western Alaska, particularly Bristol Bay, was given in Margolis (1963).

Of the five principal sockeye-producing river systems tributary to Bristol Bay (Wood, Naknek, Kvichak, Ugashik, and Egegik), *T. crassus* was found regularly in samples from the Wood and Naknek and occasionally in samples from the Ugashik and Kvichak. In the Wood, Naknek, and Ugashik systems, *T. crassus* plerocercoids were observed both in adults returning to spawn and in downstream migrants (smolts), leaving no doubt that the parasite was acquired in the watersheds in question. In the Kvichak system, on the other hand, *T. crassus* was not found in any of 125 smolts collected during the years 1956–59, but it occurred in 2 of 119 prespawning adults sampled during the same years. (Both *Triaenophorus*-infected fish were part of a sample of 49 fish taken in 1959.) Since *T. crassus* was not encountered in any of the Kvichak smolts examined, there remained the possibility that this cestode is not indigenous to the Kvichak system and that the *Triaenophorus*-infected adults returning to this system were actually "strays" from the adjacent Naknek River, or from one of the other Bristol Bay rivers.

Recently, in connection with studies on the dracunculoid nematode *Philonema oncorhynchi*, we had the opportunity to examine additional sockeye salmon smolts from the Bristol Bay area. Among these samples were some from the Kvichak River system, in which *T. crassus* was occasionally found (Table I), providing evidence that this parsite is, indeed, indigenous to the Kvichak

TABLE I. Incidence of *Triaenophorus crassus* plerocercoids in sockeye salmon smolts from the Kvichak River system.

| Date of collection | Number examined | Number with *T. crassus* |
|---|---|---|
| June 6, 1964 | 98 | 1 |
| June 8, 1964 | 100 | 0 |
| June 14, 1964 | 100 | 3 |
| May 23, 1965 | 50 | 0 |
| May 26, 1965 | 50 | 1 |
| May 29, 1965 | 50 | 0 |

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system. It is apparent from the results presented here, and those reported in 1963, that the incidence of *T. crassus* in Kvichak River sockeye salmon is very low. The intensity of infection is also low; in 6 of the 7 infected fish only one plerocercoid was found and in one case two plerocercoids were recovered.

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

L. MARGOLIS

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# Preservation of Raw Material for Fish Protein Concentrate with Isopropyl Alcohol

The procedure for the preparation of fish protein concentrate (FPC) as developed at this laboratory (Guttmann and Vandenheuvel, 1957; Dambergs, 1959) and later improved and applied to many species of fish (Power, 1962, 1964a, b) utilizes isopropyl alcohol (IPA) as the solvent to extract lipids and other substances that may cause undesirable flavors or odors. IPA has also been shown to be an effective preservative and inhibitor of lipolytic enzymes in fish livers (Guttmann and Vandenheuvel, 1958; Jangaard and Power, 1965). An investigation showed that the raw materials for FPC production, such as scrap (chiefly skeleton and attached muscle), from filleting lines can be advantageously treated with IPA as produced and stored for later processing, the IPA serving as a preservative and subsequently as the first extractant in the regular procedure.

*Scraps from filleting cod.*—Isopropyl alcohol was added to fresh, ground filleting scrap which on the average consisted of 45% bones and 55% skeletal muscle. Quantities of IPA to make a final concentration by weight of 5, 7½, 10, 12½, 15, and 17½% were added and well mixed. A small quantity of antioxidant was also added. The mixture was placed in 1-gal cans lined with polyethylene bags; the bags and cans were sealed airtight and kept at room temperature.

After 1, 2, and 3 months, cans with different IPA contents were opened, organoleptically tested, and the bacteria counted. The first two samples with respectively 5 and 7½% IPA, went bad during the first month and were discarded. The cans with 10 and 12½% IPA were organoleptically unchanged at the same time. The bacterial count was 2000/g after the first month and did not increase during the second and third months. The cans with 15 and 17½% IPA were organoleptically unchanged after 3 months and a bacteriological examination failed to demonstrate the presence of bacteria. The proteolytic enzymatic activity was negligible.

The preserved fish was processed to FPC by extracting with IPA using a solvent to water ratio near the optimum range for fat extraction (Dambergs, 1959). For example, 3000 g scrap preserved with 13% IPA contained about 1750 g skeletal muscle, 1250 g bones, and 450 g IPA. The muscle contained about 1400 g water, consequently 2150 g more IPA was added; the mixture was stirred in a Waring blendor, boiled under reflux for 20 min, and filtered while hot with the application of gentle suction. The cake was washed with 500 g IPA, then suspended in twice its weight of IPA and extracted as before. The presscake was air-dried and screened into two fractions. Both fractions were separately ground and dried under vacuum to remove the last traces of IPA. The extractions were done in duplicate and the properties of the products obtained are given in Table I. A certain amount of bones without adhering protein particles was discarded during the screening process. This explains the relatively low ash content of the final product.

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TABLE I. FPC prepared from preserved cod scraps.

| | Fraction I | Fraction II |
|---|---|---|
| Yield, % | 11.5, 12 | 4.5, 5 |
| Organoleptic test and color | Creamy white, no odor, no taste | Greyish white, no odor, no taste |
| Moisture, % | 4.0, 4.25 | 3.6, 4.0 |
| Ash, % | 8.0, 8.5 | 33, 35 |
| CaO, % | 2.5, 3.0 | 15, 16 |
| Total N %, F = 6 | 14.5, 15.0 | 9.5, 10 |
| Lipids, % | 0.05, 0.08 | 0.05, 0.10 |

The values given in Table I show that the FPC compares favorably with those prepared by Power from fresh raw material.

*Herring.*—The preservation of herring is much more difficult due to the enzymatic activity of the digestive system, especially when the fish are feeding.

Two series of experiments were carried out with ground herring, one on fish caught in June when actively feeding and in August during spawning when feeding had practically ceased. In both instances some liquification occurred on storage at room temperature. However, in some cases these concentrations of IPA (<20%) were satisfactory for short term storage (1–2 weeks). Higher concentrations of IPA as indicated by the condition of the fish and the ambient temperature could be used when longer storage is desirable.

In all experiments the lipolytic enzymes were inhibited. The amounts of free fatty acids in the oil did not go above 3.2% even when the fish was completely liquified, and in most cases stayed below 2%.

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

A. GUTTMANN[1]
N. DAMBERGS
P. M. JANGAARD

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[1] Deceased.

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